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INFLUENCE OF CULTURE CONDITIONS UPON THE GROWTH AND DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM [1]

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INTRODUCTION

Dictyostelium discoideum, a new species of the Acrasieae described by the writer (*18*),[3] was recently shown to be capable of feeding upon bacteria belonging to many different species when these were grown upon hay-infusion agar (*19*). This medium was accordingly considered admirable for investigating the "host range" of a species of the Dictyosteliaceae. Nevertheless, it left much to be desired and certain of its characters were plainly objectionable: First, it was not an optimum medium for any bacterial species studied and consequently the growth of the *Dictyostelium* was regularly limited by the restricted growth of the host bacteria; second, it was of unknown and somewhat variable composition despite all possible care in the selection of source material and in the preparation of the infusion; and third, it was light brown in color and, because of the difficulty of transmitting light through it, was not well suited for microscopic observation of myxamoebae in situ on agar plates.

With these objections in mind, the writer undertook to find a medium or media that would be free from the more obvious limitations of hay-infusion agar, and, at the same time, endeavored to study critically the combined influence of the culture medium and of the associated bacteria upon the growth and subsequent development of a species of the Dictyosteliaceae. The need for such a study was apparent. Except for the work of Potts (*17*) there has been little appreciation of the changes brought about in culture media by the growth of bacteria associated with these slime molds; and no careful effort has been made to analyze the influence of such changes on the behavior of an organism of this group. Further, species of the Dictyosteliaceae have been

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[2] The writer wishes to acknowledge his indebtedness to Prof. William H. Weston, Jr., of Harvard University, under whose direction the present study was pursued, for valuable counsel and criticism during the progress of the work; to Prof. Kenneth Thimann, likewise of Harvard University, for advice in connection with the studies of hydrogen-ion concentration; and to Dr. Charles Thom and N. R. Smith, of the Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture, for their generous cooperation in making this investigation possible.

[3] Italic numbers in parentheses refer to Literature Cited, p. 197.

found to occur commonly in soil and decaying vegetation (*20*), but surprisingly little is known of the environmental conditions that favor or retard their growth in these situations.

Because of its unique developmental behavior (*18*) and because its growth with different bacterial associates can be conveniently measured in terms of the number and size of the sorocarps produced, *Dictyostelium discoideum* is especially adapted for a study of the interrelationships between an amoeboid organism and the bacteria on which it feeds (*19*). For the present study this species was selected because, in its growth and more particularly in the development of its fruiting structures, *D. discoideum* is extremely sensitive to conditions of culture and slight changes in environment are reflected in the pattern of the sorocarps developed. Thus the favorableness of cultures to this slime mold can be measured to a degree that is not practicable with other species of this group, or, for that matter, with soil amoebae generally.

REVIEW OF LITERATURE

Students of the Dictyosteliaceae have regularly cultivated these organisms in the laboratory, and in their studies a wide variety of media have been employed. Brefeld used cooked horse dung as a solid substratum for mass cultures and a filtered dung decoction for microcultures in his pioneer work on *Dictyostelium mucoroides* Bref. (*3*), and later in his study of *Polysphondylium violaceum* Bref. (*4*). Van Tieghem employed cakes of beer yeast and drops of fresh urine for cultivating *Acrasis granulata* Van Tieghem and species of *Dictyostelium* (*25*), and later he used decaying broadbeans and urine for culturing *Coenonia denticulata* Van Tieghem (*26*). Olive (*14*), in his comprehensive study of the Acrasieae, found weak horse-dung decoction and peptone-solution agars to be the most satisfactory of the various media tested. Harper (*9*, *10*) likewise utilized a weak horse-dung decoction both as a liquid and as an agar medium for his morphogenetic studies of *Dictyostelium mucoroides* (*8*) and *Polysphondylium violaceum*. Raper and Thom (*20*) cultivated species of the Dictyosteliaceae upon hay, dung, and carrot-infusion agars and upon mannite agar to which rat dung was added. Similarly, the writer (*18*) cultivated *Dictyostelium discoideum* upon hay- and dung-infusion agar in his initial study of this species.

In all the above investigations bacteria were reported as being present in the cultures; but in no case was the identity of the accompanying bacteria known, nor was any role of importance ascribed to them. On the other hand, some investigations have been made in which the identity of the associated bacteria was known and in which a role of primary importance was attributed to them, either directly as a source of food for the slime molds (*13*, *15*, *16*, *17*, *19*, *21*, *22*, *24*, *27*) or indirectly as agents capable of altering the culture medium (*12*).

The possibility of the accompanying bacteria changing the culture medium was first recognized by Nadson (*12*), who cultivated *Dictyostelium mucoroides* upon sterilized dung, and upon gelatin and agar media containing malt, an extract of dung, or peptone and inorganic salts. He reported that *Bacillus fluorescens liquefaciens* was the habitual associate of *D. mucoroides*, and that in cultures with this bacterium the slime mold developed with an intensity and vigor equal to that under natural conditions. This led him to conclude

that the bacteria favored the growth of *Dictyostelium* by creating an alkaline medium through the production of ammonia.

Potts (*17*) cultivated *Dictyostelium mucoroides* upon sterilized dung and dung infusion, upon infusion agar made from the stems of *Vicia faba*, and upon an extract of corn (maize) grains which he employed either as a liquid or solid medium. Employing corn gelatin, which he found to be especially favorable, he observed that *Dictyostelium* developed only in the presence of bacteria, and he succeeded in isolating the slime mold with a single bacterial species, which he described as *Bacterium fimbriatum*. Using this association, he further studied the relationship existing between these organisms by cultivating them upon a large number of synthetic media, each of which contained some inorganic or organic source of nitrogen together with some sugar, organic acid, or other source of carbon. While he obviously misinterpreted the manner in which the slime mold fed upon the accompanying bacteria, believing they were digested extracellularly, he made no mistake in his conclusion that the *Dictyostelium* was dependent upon bacteria for nutriment. Furthermore, he was not unmindful of the influence the bacteria might exert through altering the culture medium, and reported that *D. mucoroides* could grow in slightly acid to strongly alkaline media but grew best in media of a mild alkaline reaction.

Employing a maltose peptone agar medium, Vuillemin (*27*) cultivated *Dictyostelium mucoroides* in association with *Bacillus fluorescens non-liquefaciens*. In the same year Pinoy (*15*) succeeded in growing *D. mucoroides* in association with *B. fluorescens liquefaciens*, *B. coli communis*, and *Microbacillus prodigiosus* upon pieces of sterilized carrot. In a more comprehensive study 4 years later, in which *D. purpureum* Olive and *Polysphondylium violaceum* as well as *D. mucoroides* were studied, and in which five additional bacterial associates for *D. mucoroides* were reported, Pinoy (*16*) employed for the most part a flaxseed-infusion agar. Again he profitably used strips of carrot, but found potato unsuitable, although it afforded a favorable medium for *B. fluorescens liquefaciens*. In addition, he reported that agar containing bouillon or peptone would not support the growth of *D. mucoroides*, although ample bacteria were present, unless lactose, maltose, or glucose was added. He apparently did not consider the possible influence of acidity or alkalinity upon the growth of the slime mold.

Skupienski (*23*, *24*) employed a weak hay infusion either as a liquid medium or incorporated in agar, and, like Nadson (*12*) and Pinoy (*16*), cultivated *Dictyostelium mucoroides* with *Bacillus fluorescens liquefaciens*. Oehler (*13*) cultivated the same slime mold upon nonnutrient agar; and while he did not report the identity of any associated bacteria, he implied that the *Dictyostelium* was grown with a number of different species. Obtaining his culture from Oehler, Schuckmann (*21*, *22*) subsequently cultured *D. mucoroides* upon dilute beef-bouillon agar in association with *B. coli communis* and two additional unidentified Gram-negative bacteria.

Hay-infusion agar alone was employed by the writer (*19*) in earlier work dealing with the growth and development of *Dictyostelium discoideum* in association with a large number of different bacterial species.

INFLUENCE OF CULTURE MEDIUM AND OF BACTERIAL ASSOCIATE

PRELIMINARY STUDIES

A number of substrata were investigated that were more or less comparable with those employed and recommended by earlier students of the Dictyosteliaceae. Such media included horse-dung-infusion agar (*3*, *4*, *8*, *9*, *10*, *12*, *14*, *17*, *21*), carrot-infusion agar (*15*, *20*), corn-meal agar (*17*), flaxseed-infusion agar (*16*), bean-infusion agar (*26*), yeast agar (*25*), nonnutrient agar (*13*), and nutrient-broth agar (*21*, *22*). None of these media proved wholly suitable, however, and each was unsatisfactory from one or more points of view. Upon dung, corn meal, flaxseed, and nonnutrient agars *Pseudomonas fluorescens* Migula [4] and *Serratia marcescens* Bizio, the species selected as bacterial associates for these preliminary studies, failed to grow as luxuriantly as desired; consequently, the growth of *Dictyostelium discoideum* was limited accordingly. Upon bean, yeast, and nutrient-broth agars the bacteria grew satisfactorily; but *D. discoideum* grew very poorly upon bean agar and failed completely to grow upon yeast and nutrient-broth media. Carrot agar alone proved reasonably satisfactory, for it supported a good growth of bacteria and subsequently of *Dictyostelium*. However, it could not be considered an especially favorable medium because, like hay-infusion agar, it was of unknown and, to a limited degree, of variable composition.

STUDIES WITH ENDO'S AND ALLIED MEDIA

Having failed to secure an entirely satisfactory culture medium among the above-named substrata, attention was next directed toward agar media in which the essential components were peptone and some type of sugar. The possibilities inherent in this combination of nutrients were first suggested to the writer by observing a culture of *Escherichia coli* (Migula) Castellani and Chamlers growing upon Endo's medium.[5] The bacteria grew well upon this medium, and from the writer's earlier studies with hay-infusion agar (*19*) *E. coli* was known to afford excellent food for *Dictyostelium discoideum*. Endo's medium was, therefore, secured for the purpose of determining whether the slime mold could grow upon it in association with *E. coli*.

The growth of *Dictyostelium discoideum* was immediate and excellent. Pseudoplasmodia and sorocarps began developing on the second day after the spores were introduced and continued to form for several days thereafter. At the end of a week the bacteria were almost wholly consumed and the growth of *Dictyostelium* had virtually ceased. Sorocarps were very abundant and were entirely normal in shape and proportions, the basal disks being circular, flat, and expanded; the sorophores were straight or slightly sinuous and evenly tapered; and the sori were large, yellow in color, and spherical to slightly elliptical in form. (See fig. 3.)

[4] In discussing the writer's work, the nomenclature for the bacterial species studied is that of Bergey (*2*), but in reviewing the work of earlier investigators, the nomenclature used by those investigators is followed.

[5] Endo's agar is almost universally used for differentiating between *Eberthella typhosa* (Zopf) Weldin (*Bacillus typhosus* Zopf) and *Escherichia coli* (*Bacillus coli communis* Steinberg). The essential ingredients of this medium are lactose and peptone as nutrient materials, fuchsin decolorized with sodium sulphite as an indicator, and usually sodium carbonate or dipotassium phosphate to render the medium alkaline. When *E. coli* is grown upon this medium the lactose is broken down with the consequent production of aldehydes, and hence the bacterial colonies and the medium underlying them become deep red in color.

Since a medium of known [6] and easily reproducible composition had been found upon which *Dictyostelium discoideum* grew luxuriantly and developed normally in association with *Escherichia coli*, it now became desirable to make a thorough study of this medium and to determine just what components were essential to obtain a good growth of *E. coli* and subsequently of *D. discoideum;* and conversely, what, if any, ingredients were nonessential. Similarly, it was desirable to determine whether Endo's medium would be equally applicable to another combination of slime mold and bacteria, e. g., *D. discoideum* in association with *Pseudomonas fluorescens*.

To accomplish these ends a series of media was prepared according to an Endo's formula, except that in each a single and different constituent, barring agar and distilled water, was omitted. Thus one medium was a complete Endo's medium except for the presence of peptone, a second complete except for the presence of lactose, etc. Since the Endo's medium first employed was a Difco [7] preparation, the formula used by that organization was employed as a basis for compounding the series of media (*7*).

In addition to the above, three other media were prepared and studied, namely, a complete Endo's medium; a medium of the same nutrient composition (1.0 percent each of peptone and lactose) but without sulphite and with eosin and methylene blue substituted for basic fuchsin; [8] and a medium which contained 1.2 percent of lactose and 0.4 percent of peptone but neither inorganic salts nor dyes.

Duplicate Petri plates of the several media were inoculated with *Escherichia coli*, five colonies being established per plate. At the same time, duplicate plates of the complete Endo's medium, the eosin-methylene blue medium, and the lactose-peptone medium were similarly inoculated with *Pseudomonas fluorescens*. Pure spores of *Dictyostelium discoideum*, obtained in the manner described in an earlier paper (*19*), were introduced into the resulting colonies of bacteria at the end of 2 days. Incubation throughout the experiment was at 20° to 23° C.

Within 2½ days after the introduction of pure spores of *Dictyostelium discoideum*, the central areas of all bacterial colonies had been cleared by the feeding and growing myxamoebae; and in all cultures with *Escherichia coli*, except in colonies on the Endo's medium minus lactose, pseudoplasmodia were present and young sorocarps were developing. In colonies of *Pseudomonas fluorescens* there was evidence of growth and multiplication of myxamoebae, but the cultures were plainly less vigorous than those with *E. coli* on identical media.

The growth and development of *Dictyostelium discoideum* in rich and favorable bacterial colonies such as those of *Escherichia coli* upon media containing both peptone and lactose (fig. 1) was in all cases much more luxuriant than reported previously for hay-infusion agar (*19*), and, in general, proceeded in the following manner. Within 20 to 24 hours after the spores were added, the first macroscopic sign of growth of the myxamoebae became evident as small centrally placed areas in which the bacteria had been noticeably consumed. During

[6] It is, of course, recognized that peptones vary both in composition and in nutritive value, but this difficulty can be reduced to a minimum by using a product of high purity, and by using the same brand of peptone for all experiments where any comparison of results is anticipated. In this and in all succeeding experiments, the writer employed a peptone prepared and marketed by the Digestive Ferments Co. under the trade name "Bacto-Peptone."

[7] Product of the Digestive Ferments Co.

[8] A medium prepared according to a formula published by Levine (*11*).

the following day these areas were gradually extended and when viewed under a low-power binocular microscope appeared finely and distinctly punctate owing to the refraction of light by the countless myxamoebae present. When the bacteria had been completely consumed in the colony centers, the myxamoebae commenced to form pseudoplasmodia (fig. 1, *A*, *b*). These did not form by the inflowing of long, extended, and ramifying streams of myxamoebae, as had

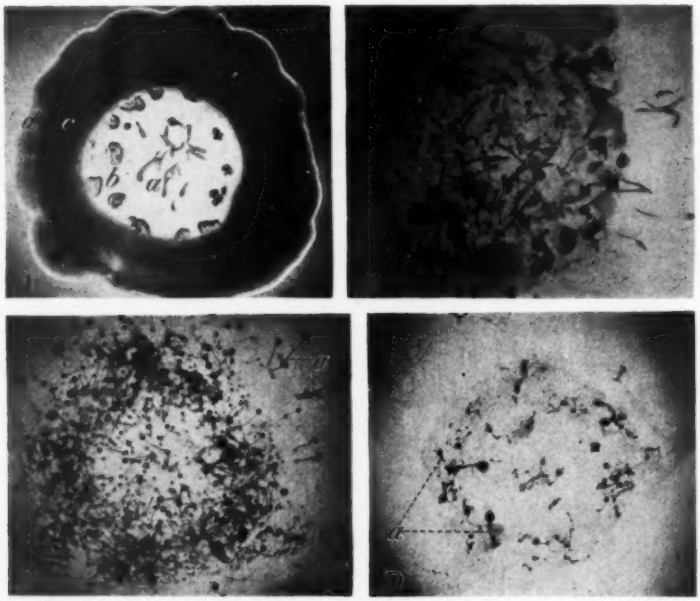

FIGURE 1.—Growth of *Dictyostelium discoideum* in association with *Escherichia coli* and *Pseudomonas fluorescens* upon media containing lactose and peptone. *A*, 2-day-old culture of *Dictyostelium* with *E. coli* upon eosin-methylene blue agar; *a*, Pseudoplasmodia ready to begin migration; *b*, forming pseudoplasmodia; *c*, zone in which myxamoebae are actively feeding; *d*, zone into which myxamoebae have not yet advanced. × 3. *B*, 3½-day-old culture with *E. coli* upon lactose (1.2 percent)-peptone (0.4 percent) medium; bacterial colony almost wholly consumed by myxamoebae which for the most part have now formed pseudoplasmodia or sorocarps. × 3. *C*, 8-day-old culture with *E. coli* upon Endo's medium minus fuchsin; bacterial colony wholly consumed by myxamoebae which subsequently formed (*a*) sorocarps of normal pattern. × 3. *D*, 8-day-old culture with *Pseudomonas fluorescens* upon eosin-methylene blue agar; bacterial colony consumed but produced (*a*) sorocarps of abnormal pattern, indicating presence of conditions not entirely favorable to the slime mold. × 3. (Photographs by Frank White.)

previously been described as typical of this and related slime molds, but rather by a blocking off of the myxamoebae into irregular masses that subsequently evolved into elongate, cylindrical pseudoplasmodia that in form and size were characteristic of *D. discoideum* (fig. 1, *A*, *a*). At the same time, adjacent to and surrounding these centrally cleared areas were zones in which the myxamoebae were actively

feeding and vegetating (fig. 1, *A*, *c*). In such colonies, therefore, the vegetative stage (i. e., the growth and multiplication of individual and separate myxamoebae) and the fruiting stage (i. e., the organization of

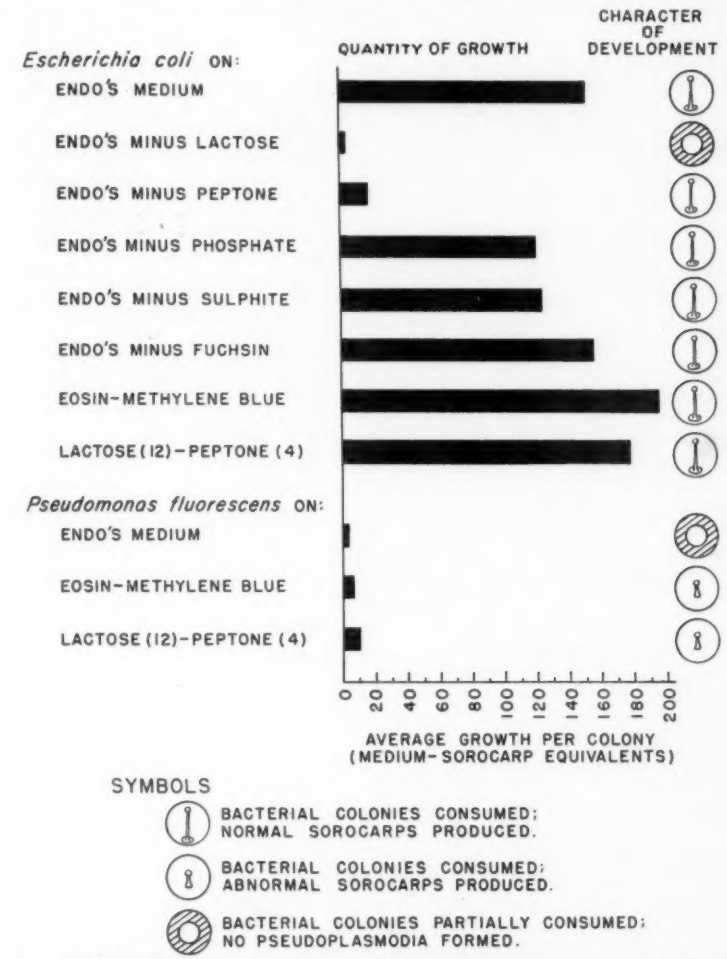

FIGURE 2.—Comparison of the amount of growth and the character of development of *Dictyostelium discoideum* in association with *Escherichia coli* and *Pseudomonas fluorescens* on Endo's and allied media.

myxamoebae into pseudoplasmodia and the subsequent formation of sorocarps) proceeded simultaneously and almost side by side; and as the former phase moved toward the colony edge the latter followed in its wake approximately 24 hours later.

A comparison of the total growth and of the character of development of the slime mold upon the different media and in association with the two bacterial hosts is presented in figure 2. For this comparison the average total number of large, medium, and small sorocarps per bacterial colony is expressed in the average number of medium-sorocarp equivalents, the necessary conversions being made by the method employed in earlier work (*19*). In this study it was found that the proportion of stalk or sorophore, to spore mass, or sorus, remained essentially the same regardless of the size of the sorocarp; therefore, by computing the volumes of their sori, sorocarps of different size could be readily represented in terms of a common unit such as a medium-sized sorocarp, for which unit the term "medium-sorocarp equivalent" was introduced.

Some striking and suggestive results were obtained from this experiment. *Dictyostelium discoideum* grew luxuriantly and developed normally in colonies of *Escherichia coli* only upon those media that contained both lactose and peptone. In the absence of peptone the growth of the bacterial associate was slight; and although the bacteria were completely consumed by the myxamoebae, the growth of *Dictyostelium* was correspondingly meager (fig. 2). On the other hand, *E. coli* grew excellently in the absence of lactose, but the myxamoebae grew very poorly and for a short time only, indicating that something inimical to the growth of the slime mold was being produced in these cultures (fig. 2). Phosphate and sulphite, additional ingredients of the complete Endo's medium, were found to be unnecessary, and through the omission of fuchsin a medium was secured that was equal to Endo's medium in every particular, and in the absence of color was markedly superior. That lactose and peptone were the essential ingredients of a favorable medium was clearly demonstrated by the excellent growth of *E. coli* and by the subsequent growth and normal development of *D. discoideum* which occurred upon the medium containing these components alone (fig. 2).

Equally indicative results were obtained from the cultures with *Pseudomonas fluorescens*. This bacterium flourished upon Endo's medium, but the slime mold grew poorly and but for a limited period, and only the central areas of the bacterial colonies were cleared by the myxamoebae. Thus these cultures were strikingly similar to those previously noted with *Escherichia coli* upon the lactose-free medium (fig. 2). Further, the cultures with *Ps. fluorescens* on the eosin-methylene blue medium and on the lactose-peptone medium presented evidence of unfavorable conditions approaching the above (fig. 2).

When it was recalled that *E. coli* could ferment lactose and *Ps. fluorescens* could not, the results of this experiment immediately suggested the following hypothesis: For the successful cultivation of *Dictyostelium discoideum* upon a peptone-rich medium, the peptone must be accompanied by a sugar that the associated bacteria can ferment. Furthermore, a balanced fermentation of the two nutritive ingredients of the medium must take place; that is, the carbon source, yielding acid products, and the nitrogen source, yielding alkaline products, must be broken down simultaneously, and the products of this dual fermentation must counteract one another.

STUDIES WITH PEPTONE-SUGAR MEDIA

Preliminary experiments designed to test the above-mentioned hypothesis supported its correctness, for it was uniformly found that upon media containing peptone and some sugar, good growth and normal development of *Dictyostelium discoideum* were limited to cultures in which the bacterial associate could ferment the carbohydrate contained in the medium. However, continued study along this line was considered worth while in order to understand adequately the combined influence of the composition of the culture medium and of the fermentations of the associated bacteria in their relation to the growth and development of *Dictyostelium*.

Media of the following composition were prepared with distilled water and solidified with 1.5 percent of agar: (1) A series of four media containing 1, 2, 4, and 12 g of peptone per liter as the sole nutrient; (2) a series of four media containing 12 g of dextrose and 1, 2, 4, and 12 g of peptone per liter as nutrients; (3) a series of four media containing 12 g of lactose and 1, 2, 4, and 12 g of peptone per liter as nutrients; and (4) a series of four media containing 12 g of sucrose and 1, 2, 4, and 12 g of peptone per liter as nutrients.

The following species of bacteria were selected for this investigation upon the basis of their sugar fermentations: *Pseudomonas fluorescens*, No. 112,[9] which ferments dextrose but not lactose or sucrose; *Escherichia coli*, No. 107, which ferments dextrose and lactose but not sucrose; and *Escherichia communior* (Jackson) Bergey et al., No. 102, which ferments dextrose, lactose, and sucrose.

Duplicate Petri plates of the various media were inoculated with the selected bacteria, five colonies being planted in each plate. At the end of 3 days pure spores of *Dictyostelium discoideum* were introduced into four of the five colonies in each plate, the uninoculated fifth being kept as a measure of the bacterial growth. The cultures were incubated at 18° to 22° C. throughout the experiment.

A record of the growth of the three bacteria upon the several media was made at the time the spores of *D. discoideum* were introduced; and a tabulation of the quantity of growth and the character of development of *Dictyostelium* in colonies of the different bacteria upon the various media was made on the eighth or ninth day after the introduction of the spores. These records are summarized and presented in table 1.

A brief explanation of the terms used in table 1 is needed. The growth of bacteria upon the various media is listed first for each culture and is recorded in terms designated by the symbol *C* for a standard growth of *Escherichia coli* upon a medium containing 12 g of lactose and 4 g of peptone per liter, greater or less bacterial growth being denoted by a plus or minus sign, respectively. In these columns "growth" refers to the relative abundance of myxamoebae present, as evidenced by the extent to which the bacterial colonies were cleared or by the number of sorocarps produced. Colonies referred to are in all cases bacterial colonies, and the extent to which they were consumed by the myxamoebae is denoted by the degree of gross clearance. The character of sorocarps is given as normal and abnormal (fig. 3).

[9] The carbohydrate fermentations of these particular cultures, together with several others used later in this investigation, were known from the unpublished work of N. R. Smith, senior bacteriologist in the Bureau of Plant Industry, from whom the cultures were obtained.

The growth of *Dictyostelium discoideum* in colonies of the three associated bacteria will be considered separately: (1) *Dictyostelium discoideum* grew moderately well in colonies of *Pseudomonas fluorescens* on media containing dextrose but not on media containing peptone alone or peptone together with lactose or sucrose. This fact becomes particularly interesting when it is recalled that dextrose is

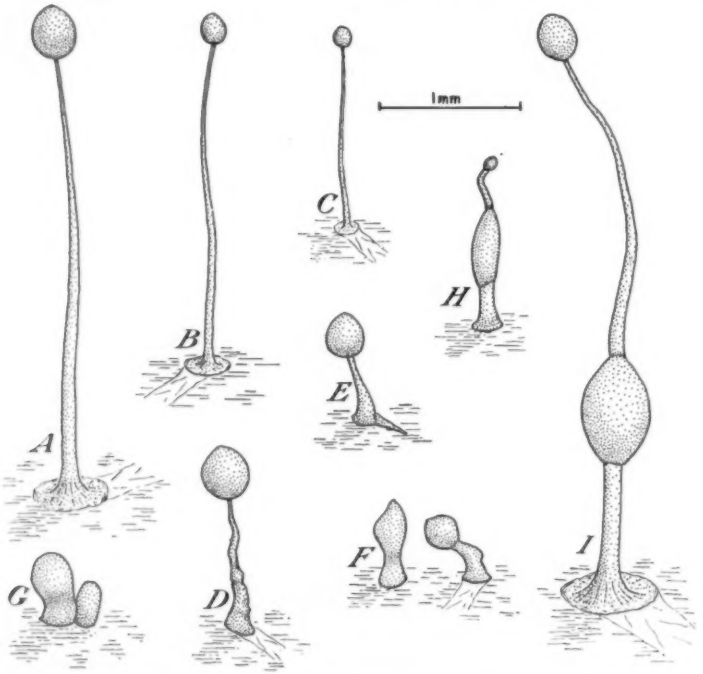

FIGURE 3.—Fruiting structures, or sorocarps, of *Dictyostelium discoideum*. *A–C*, Normal sorocarps of large (*A*), medium (*B*), and small (*C*) size; the ellipsoid head, or sorus, the upright, evenly tapering stalk, or sorophore, and the discoid base are clearly evident. *D–I*, Abnormal sorocarps: *D* and *E*, Sorocarps with relatively large sori, short, strongly tapering sorophores, and poorly formed basal disks; *F*, sorocarps with poorly differentiated sori and sorophores; *G*, masses of myxamoebae in which sori and sorophores have not been differentiated because of extremely unfavorable culture conditions; *H*, sorocarp of very abnormal form in which spores have not been produced; *I*, sorocarp in which the greater portion of the spore-forming myxamoebae produced a sorus a little below midway of the completed sorophore.

the only one of the three sugars employed that could be fermented by *Ps. fluorescens*. (2) Moderate to very good growth and normal development of *D. discoideum* occurred in colonies of *Escherichia coli* on media containing peptone and dextrose and peptone and lactose, but not on peptone alone or peptone and sucrose. Thus with *E. coli*, as with *Ps. fluorescens*, good growth and normal development of *Dictyostelium* occurred only on media containing sugars that could

be utilized by the bacteria. (3) Moderate to very good growth and normal development of *D. discoideum* took place in colonies of *Escherichia communior* on all media except that containing peptone alone. Here again good growth and normal development of *D. discoideum* could be directly correlated with the specific sugar fermentations of the bacterial culture, for *E. communior* is able to utilize dextrose, lactose, and sucrose.

TABLE 1.—*Combined influence of the bacterial associate and of the medium upon the growth and development of Dictyostelium discoideum*

Designation and composition of medium (grams per liter)	Growth [1] of *Dictyostelium discoideum* with indicated bacterial associate		
	Pseudomonas fluorescens No. 112	*Escherichia coli* No. 107	*Escherichia communior* No. 102
P1 (peptone 1, water 1,000, agar 15)	*C*−. Growth poor; colonies cleared; sorocarps slightly abnormal.	*C*−. Growth fair; colonies cleared; sorocarps near normal.	
P2 (peptone 2 : 1,000).	*C*−. Growth poor; colonies cleared; sorocarps abnormal.	*C*. Growth fair; colonies cleared; sorocarps few, abnormal.	
P4 (peptone 4 : 1,000).	*C*−. Growth poor; colonies partially cleared; few pseudoplasmodia, no sorocarps.	*C*. Growth fair; colonies cleared; sorocarps few, abnormal.	*C*. Growth poor; colonies cleared; sorocarps few, abnormal.
P12 (peptone 12 : 1,000).	*C*. No growth; spores ungerminated.	*C*+. Growth fair; colonies partially cleared; few pseudoplasmodia; no sorocarps.	*C*+. Growth very poor; colonies partially cleared; no pseudoplasmodia, no sorocarps.
D1 (dextrose 12, peptone 1 : 1,000).	*C*−. Growth fair; colonies largely cleared; sorocarps normal.	*C*−. Growth fair; colonies cleared; sorocarps normal.	
D2 (dextrose 12, peptone 2 : 1,000).	*C*−. Growth fair; colonies largely cleared; sorocarps normal.	*C*−. Growth fair; colonies cleared; sorocarps normal.	
D4 (dextrose 12, peptone 4 : 1,000).	*C*. Growth fair; colonies largely cleared; sorocarps normal.	*C*. Growth good; colonies cleared; sorocarps normal.	*C*. Growth good; colonies cleared; sorocarps normal.
D12 (dextrose 12, peptone 12 : 1,000).	*C*+. Growth good; colonies partially cleared; sorocarps normal.	*C*+. Growth very good; colonies cleared; sorocarps normal.	*C*+. Growth very good; colonies cleared; sorocarps normal.
L1 (lactose 12, peptone 1 : 1,000).	*C*−−. Growth fair; colonies cleared; sorocarps normal.	*C*−. Growth fair; colonies cleared; sorocarps normal.	
L2 (lactose 12, peptone 2 : 1,000).	*C*−. Growth fair; colonies cleared; sorocarps normal and abnormal.	*C*. Growth fair; colonies cleared; sorocarps normal.	
L4 (lactose 12, peptone 4 : 1,000).	*C*−. Growth poor; colonies largely cleared; sorocarps abnormal.	*C*. Growth good; colonies cleared; sorocarps normal.	*C*. Growth good; colonies cleared; sorocarps normal.
L12 (lactose 12, peptone 12 : 1,000).	*C*. No growth; spores ungerminated.	*C*+. Growth very good; colonies cleared; sorocarps normal.	*C*+. Growth very good; colonies cleared; sorocarps normal.
S1 (sucrose 12, peptone 1 : 1,000).	*C*−−. Growth fair; colonies cleared; sorocarps normal.	*C*−−. Growth fair; colonies cleared; sorocarps normal.	*C*−. Growth poor; colonies cleared; sorocarps normal.
S2 (sucrose 12, peptone 2 : 1,000).	*C*−. Growth fair; colonies cleared; sorocarps few, abnormal.	*C*−. Growth fair; colonies cleared; sorocarps abnormal.	*C*−. Growth fair; colonies cleared; sorocarps mostly normal.
S4 (sucrose 12, peptone 4 : 1,000).	*C*−. Growth fair; colonies cleared; pseudoplasmodia; very few sorocarps, abnormal.	*C*−. Growth fair; colonies largely cleared; sorocarps few, abnormal.	*C*. Growth good; colonies cleared; sorocarps mostly normal.
S12 (sucrose 12, peptone 12 : 1,000).	*C*. No growth. Spores ungerminated.	*C*. Growth fair; colonies partially cleared; no sorocarps.	*C*+. Growth very good; colonies cleared; sorocarps normal.

[1] See text for fuller explanation of terms used.

Turning now from the growth of *Dictyostelium discoideum* with specific bacteria to more general considerations, some additional points are worthy of attention. (1) Although the presence of an available sugar in the medium did not lead to an appreciable increase

in the amount of bacterial growth (table 1), its decomposition by the bacteria obviously supplied something which rendered the bacterial colonies favorable to the slime mold, for in no case did good growth and normal development of *D. discoideum* occur where the peptone was not accompanied by a sugar that could be broken down by the associated bacteria (fig. 4). (2) In cultures where the sugar could be utilized by the associated bacteria, the amount of growth of *Dictyostelium* increased progressively with the concentration of peptone, and, therefore, as the quantity of bacterial growth increased. Conversely, in cultures where there was no sugar, or an unavailable

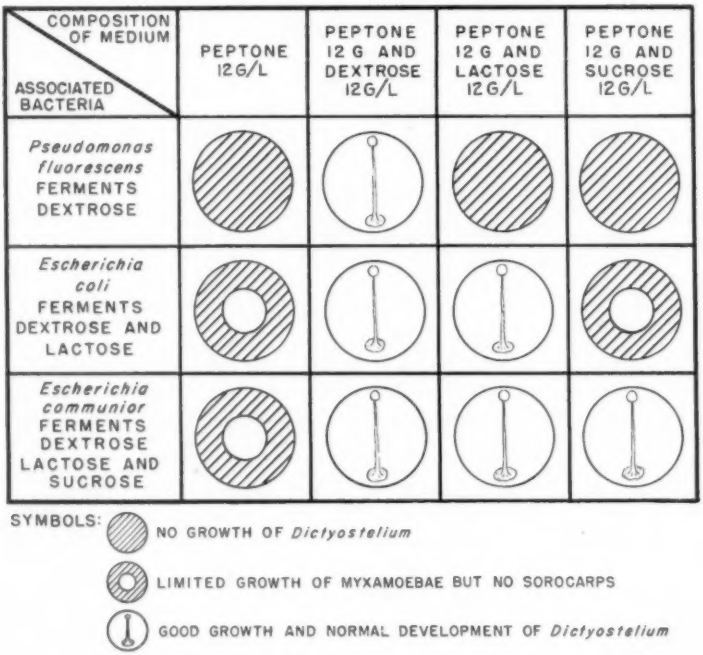
COMPOSITION OF MEDIUM
ASSOCIATED BACTERIA
PEPTONE 12G/L
PEPTONE 12 G AND DEXTROSE 12G/L
PEPTONE 12 G AND LACTOSE 12G/L
PEPTONE 12 G AND SUCROSE 12G/L
Pseudomonas fluorescens FERMENTS DEXTROSE
Escherichia coli FERMENTS DEXTROSE AND LACTOSE
Escherichia communior FERMENTS DEXTROSE LACTOSE AND SUCROSE
SYMBOLS:
NO GROWTH OF Dictyostelium
LIMITED GROWTH OF MYXAMOEBAE BUT NO SOROCARPS
GOOD GROWTH AND NORMAL DEVELOPMENT OF Dictyostelium

FIGURE 4.—Diagram illustrating the combined influence of the medium and of the bacterial associate upon the growth and development of *Dictyostelium discoideum*.

sugar, the growth of the slime mold decreased progressively with an increase in peptone, and therefore with an increase in bacterial growth (table 1). (3) Good growth and normal development of *D. discoideum* did not occur in colonies of any of the three bacteria upon media containing only peptone or peptone and an unavailable sugar. However, more nearly normal development occurred upon low than upon higher concentrations of peptone (table 1). This clearly indicated that with increasing amounts of peptone, and in the absence of fermentable sugars, the associated bacteria produced some substance inimical to the growth and normal development of *Dictyostelium* in progressively greater quantity. (4) In cultures upon media con-

taining peptone and an available sugar, the growth of *Dictyostelium discoideum* was proportional to the quantity of bacteria present and was limited solely by the abundance of the food supply; whereas, in cultures upon media containing peptone alone or in conjunction with an unfermentable sugar, factors other than the abundance of the food supply governed the growth of the slime mold, and in these latter cultures it was invariably observed that the character of development (i. e., the presence or absence of sorocarps, or the form of sorocarps produced) afforded a reliable measure of the favorableness or unfavorableness of culture conditions to *D. discoideum* (p. 189–190).

After the completion of the work just described, a supplementary experiment wholly substantiated the results already obtained regarding the influence of the bacterial associate and of the medium upon the growth and development of *Dictyostelium discoideum*. Duplicate plates of a medium containing 12 g of peptone and 12 g of soluble starch per liter were inoculated with *Pseudomonas fluorescens, Escherichia coli, E. communior,* and *Cellulomonas liquata* (Kellerman et al.) Bergey et al. Of these species only the latter is able to hydrolyze starch. Pure spores of *D. discoideum* were introduced into the bacterial colonies when 2 days old. *Dictyostelium* failed to grow in colonies of *Ps. fluorescens;* grew poorly but failed to form pseudoplasmodia and sorocarps in colonies of *E. coli* and *E. communior;* but grew well and produced normal, well-formed sorocarps (fig. 3, *A*, *C*) in colonies of *C. liquata*. Thus, another case was presented where the presence of good growth and normal development could be traced to the ability of the associated bacteria to ferment the carbohydrate contained in the medium.

For the successful cultivation of *Dictyostelium discoideum* upon peptone-sugar media the importance of a balanced fermentation of the component nutrients by the associated bacteria cannot be overemphasized. In the experiments just considered, the writer employed media containing up to 1.2 percent each of peptone and some carbohydrate fermentable by the accompanying bacteria. However, this concentration of nutrients was chosen arbitrarily, and supplementary experiments have shown that media containing 1 percent each of peptone and a fermentable carbohydrate yield equally satisfactory results. Furthermore, the concentrations may be augmented if the nutrients are increased proportionately, e. g., luxuriant growth and normal development of *D. discoideum* occur in association with *Escherichia coli* upon media containing 2.5 percent each of peptone and lactose, whereas poor growth and abnormal development occur where the concentration of peptone is increased above 1.2 percent without a corresponding increase in lactose.

Finally, a point deserving particular attention was brought out in certain of the writer's cultures through their occasional contamination with fungi belonging to the genera *Penicillium* and *Aspergillus*. It has been repeatedly noted that the growth of *Dictyostelium discoideum* was limited or wholly inhibited in colonies of bacteria grown upon media containing peptone and some sugar which was unavailable to the associated bacteria (fig. 4). However, when fungus contaminants occurred in such cultures, *D. discoideum* grew well and developed normally in colonies of bacteria adjacent to the fungus colonies. Since it was known that these fungi were able to break down the sugars con-

tained in the media, the following explanation was suggested: In breaking down the sugars the fungi produced something which counteracted the toxic products formed from the peptone by the bacteria; for in these cultures the formation of such toxic products could not have been inhibited by the fungi, since they came in at the time of inoculation with *Dictyostelium* and after the bacteria had virtually ceased growing. Further, since it was known that the bacteria in question in breaking down peptone produced ammonia and that this in turn rendered the medium alkaline, and since, on the other hand, it was known that the fungi in breaking down sugars produced acids which neutralized the ammonia and thus tended to keep the medium from becoming alkaline, it became apparent that the toxic condition existing in the bacterial colonies might well consist of an alkaline reaction resulting from the production and accumulation of ammonia.

Additional observations lent supporting evidence to this hypothesis, namely: (1) Colonies of bacteria grown upon rich peptone media are toxic to *Dictyostelium discoideum*, whereas the bacterial cells which comprise such colonies are nontoxic, thus indicating that the noxious principle, like ammonia, is a diffusible substance liberated from the peptone as the result of bacterial growth; (2) colonies of bacteria become increasingly toxic as the concentration of peptone is augmented in the absence of a fermentable carbohydrate, whereas they remain uniformly nontoxic as the percentage of peptone is increased in the presence of such a carbohydrate (table 1). Preliminary tests supported the hypothesis, for it was found that the reaction of bacterial colonies in which good growth and normal development of *D. discoideum* occurred was slightly acid (pH 6.5±), whereas the reaction of colonies supporting very limited growth of the slime mold was alkaline (pH 8.0±).

INFLUENCE OF HYDROGEN-ION CONCENTRATION

The results of the foregoing studies suggested that bacterial colonies of an alkaline reaction were toxic to *Dictyostelium discoideum*, whereas colonies of a neutral or acid reaction favored its growth and development; or, stated in another manner, they indicated that good growth and normal development of the slime mold were dependent upon the existence of a favorable hydrogen-ion concentration in the colonies of host bacteria. Since such a hypothesis lent itself readily to investigation, a series of experiments was subsequently performed for the specific purpose of determining the relationship between hydrogen-ion concentration and the culture conditions favoring optimum growth and development of *D. discoideum*.

METHODS

In these experiments the determination of the hydrogen-ion concentration of bacterial colonies on the culture plate constituted a most important phase of the investigations; and since the same procedure was followed in all experiments, a brief review of the simple colorimetric method employed for these determinations will be given forthwith. A preliminary test was first made with bromothymol blue to determine whether the colony and the adjacent medium were acid or alkaline in reaction, and once this fact was established, drops of other indicators with ranges covering the suspected pH were added. Two

or more indicators were used in making each determination, and by comparing the pH values signified by the different indicators an approximation of the true pH value was obtained. It is recognized that this method could not give exact pH readings, but the values obtained and recorded in the following experiments are believed to be correct to within approximately 0.2± of a pH unit, which was considered sufficiently accurate for the purpose of these experiments.

It should be remembered that in cultures of bacteria, or bacteria and *Dictyostelium*, the pH varies appreciably in different zones within the culture plate. It is evident that if bacteria are breaking down peptone and liberating ammonia the reaction within the colonies will be more alkaline than at some distance away. And, conversely, if the sugar is broken down rapidly the reaction within the colonies will be more acid than in areas removed from them. However, satisfactory determinations could not be made regularly within the bacterial colonies because of their opacity, uneven contours, and, in some cases, color. Nor could they be made satisfactorily in colonies of bacteria and *Dictyostelium* because of the irregularity with which the drops of indicator spread owing to the presence of the fruiting structures of the slime mold. Therefore, in all cases, except where otherwise specifically noted, the pH values recorded in the following experiments are for zones of approximately 1 cm in width adjacent to the colonies of bacteria, or bacteria and *Dictyostelium*, as the case may be.

ADDITION OF FUNGI

In an earlier experiment it was noted that upon peptone-sugar media *Dictyostelium discoideum* grew and developed normally in cultures contaminated by fungi, whereas it failed to grow in uncontaminated but otherwise similar cultures. A likely explanation of this phenomenon was believed to reside in a difference in the hydrogen-ion concentration of bacterial colonies in the presence and in the absence of fungi. Accordingly, an experiment was undertaken to determine the difference in hydrogen-ion concentration between favorable cultures in the presence of fungi and of unfavorable ones in their absence, and to investigate generally the relationship between the members of this triple association of *Dictyostelium*, bacteria, and fungi.

For this investigation agar media containing 1.2 percent each of peptone and lactose, peptone and sucrose, and peptone and starch were prepared, the initial reaction of each medium being pH 6.8 to 7.0. In order to follow the pH changes resulting from the growth of bacteria and fungi, a nontoxic indicator, bromocresol purple,[10] was incorporated in the media.

Two host bacteria were selected upon the basis of their carbohydrate fermentations, namely: *Pseudomonas fluorescens*, which ferments neither lactose nor sucrose and does not hydrolyze starch; and *Escherichia coli*, which ferments lactose but not sucrose and does not hydrolyze starch. *Penicillium chrysogenum* Thom was selected as a fungus associate because of its known vigor and its ability to utilize each of the carbohydrates contained in the media.

Plates of the different media were inoculated with bacteria as shown in figure 5. Two days later the resulting colonies were seeded

[10] Bromocresol purple has a pH range of 5.2 to 6.8 and a color change from yellow to purple. It was used in a concentration of 20 cc of a 0.04 percent aqueous solution per liter.

with pure spores of *Dictyostelium*, and immediately thereafter *Penicillium chrysogenum* was introduced into certain colonies by streaking spores along lines parallel to the rows of bacterial colonies, while no fungus was introduced into other cultures kept as controls.

A record of the growth of *Dictyostelium discoideum* in the different cultures was made on the tenth day after its introduction and is presented in table 2.

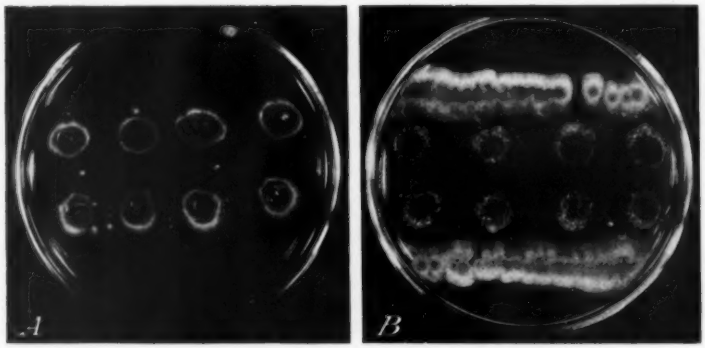

FIGURE 5.—Influence of an added fungus, *Penicillium chrysogenum*, upon the growth of *Dictyostelium discoideum* in association with *Escherichia coli* on peptone-sucrose media: *A*, No growth of *Dictyostelium* in the absence of the fungus; *B*, good growth of the slime mold in the presence of the fungus. Age: Bacterial colonies 7 days, *Dictyostelium* and *Penicillium* 5 days. × ½. (Photographs by Frank White.)

TABLE 2.—*Growth of Dictyostelium discoideum in the presence and in the absence of an added fungus, Penicillium chrysogenum*

Bacterial associate	Fungus added	Composition of medium (grams per liter)	Growth of *Dictyostelium discoideum*		
			Growth of myxamoebae	Clearance of bacterial colony	Sorocarp formation
Pseudomonas fluorescens.	*Penicillium chrysogenum.*	Peptone, 12; lactose, 12.	Good	Largely cleared	Abundant sorocarps, mostly normal in size and pattern.
	do	Peptone, 12; sucrose, 12.	do	do	Abundant sorocarps, mostly normal.
	None	Peptone, 12; lactose, 12.	None	None	None.
	do	Peptone, 12; sucrose, 12.	do	do	Do.
Escherichia coli.	*Penicillium chrysogenum.*	do	Very good.	Cleared	Very abundant, normal.
	do	Peptone, 12; starch, 12.	Good	Largely cleared	Abundant, mostly normal.
	None	Peptone, 12; sucrose, 12.	None	None	None.
	do	Peptone, 12; starch, 12.	Slight	Slightly cleared	Very few sorocarps, very abnormal.

The results of the experiment can be summarized as follows: *Dictyostelium* grew well in colonies of *Pseudomonas fluorescens* on lactose-peptone and sucrose-peptone media in the presence of *Penicil-*

lium chrysogenum, but failed to grow in colonies of this bacterium on the same media in the absence of the fungus. Similarly, good growth and normal fructification of *Dictyostelium* occurred in colonies of *Escherichia coli* on sucrose-peptone (fig. 5, *B*) and starch-peptone media in the presence of *Penicillium*, but in its absence *Dictyostelium* grew poorly or not at all (fig. 5, *A*). Thus, in each case, good growth and normal development of the slime mold could be attributed to the favorable influence of the added fungus. The following observations indicate that this influence was exerted through the creation of an acid reaction in the medium and in the colonies of host bacteria. The media were originally purple in color owing to their neutral reaction and the presence of the indicator, and they remained so, becoming more alkaline as the bacteria grew, until the added fungus began growing. As this occurred, however, acid was produced and a zone of yellow appeared, developing first directly along the line of fungus growth but subsequently spreading outward in advance of the hyphal tips. As the acid zone approached the bacterial colonies, *Dictyostelium* began to grow, and by the time the zone reached the colonies most of the bacteria had been consumed, pseudoplasmodia had formed, and normal sorocarps were developing. In cultures to which no fungus was added, the media remained purple throughout the experiment, indicating that the reaction either remained neutral or became alkaline. Subsequent tests in cultures on sucrose-peptone media without bromocresol purple showed that in the absence of fungi the reaction in colonies of *Escherichia coli* reached pH 8.0 to 8.2, and in those of *Pseudomonas fluorescens* it reached pH 8.4 to 8.6; and in neither case did the slime mold grow. Thus, in the colonies of host bacteria, the absence of good growth and normal development of *Dictyostelium discoideum* could be correlated with an alkaline reaction, whereas their presence could be correlated with an acid reaction.

The present study disclosed another interesting point. Since *Dictyostelium discoideum* grows very poorly or not at all in the absence of bacteria (*19*), when studying this slime mold or related forms one must of necessity deal with an association of two organisms; and in such a dual association the bacteria serve as hosts for the *Dictyostelium* (*19*). In the present case, however, three organisms were involved; and although the bacteria still acted as hosts for the slime mold they did so only in the presence of a third organism, a fungus, which entered the association indirectly by altering the reaction of the bacterial colonies through changing the culture medium. The bacteria and the fungus obviously acted upon the media in separate and different capacities, and the subsequent growth and normal development of *Dictyostelium* depended upon the acid products formed from the carbohydrate by the fungus counteracting the alkaline products liberated from the peptone by the bacteria. When only the peptone was broken down, ammonia accumulated, the bacterial colonies became quite alkaline in reaction, and the growth of *Dictyostelium* was inhibited; whereas, when the carbohydrate was subsequently broken down, the reaction of the bacterial colonies shifted from alkaline to acid, and in this new environment the slime mold was able to feed, grow, and form normal sorocarps. Thus was demonstrated a striking example of "micro-ecology," or the interrelationships of micro-organisms in culture.

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ADDITION OF STERILE ACID

Earlier studies indicated that good growth and normal development of *Dictyostelium discoideum* were dependent upon the existence of an acid reaction in the colonies of associated bacteria and that the toxicity of unfavorable colonies consisted in their alkaline reaction. In the preceding experiment such an acid reaction was established by the fermentation of carbohydrates by a fungus which was introduced into the cultures for that specific purpose. In the experiments next to be considered, it was obtained by the addition of free acid to cultures in which bacteria had already grown. Thus the influence of hydrogen-

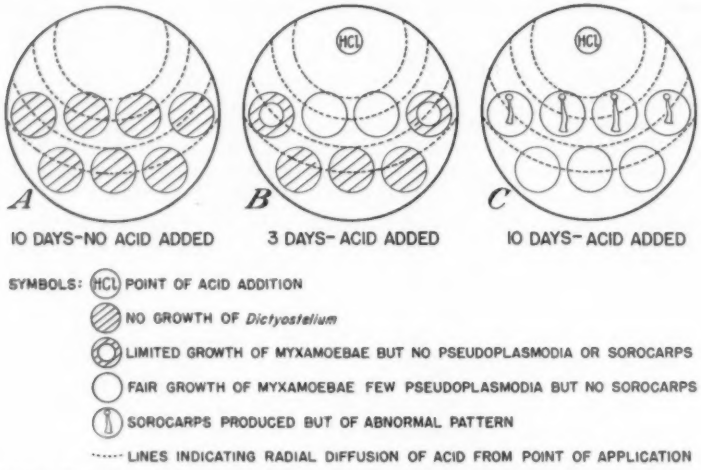

HCl
HCl
A
B
C
10 DAYS-NO ACID ADDED
3 DAYS-ACID ADDED
10 DAYS-ACID ADDED
SYMBOLS: HCl POINT OF ACID ADDITION
NO GROWTH OF Dictyostelium
LIMITED GROWTH OF MYXAMOEBAE BUT NO PSEUDOPLASMODIA OR SOROCARPS
FAIR GROWTH OF MYXAMOEBAE FEW PSEUDOPLASMODIA BUT NO SOROCARPS
SOROCARPS PRODUCED BUT OF ABNORMAL PATTERN
LINES INDICATING RADIAL DIFFUSION OF ACID FROM POINT OF APPLICATION

FIGURE 6.—The influence of added acid upon the growth of *Dictyostelium discoideum* in colonies of *Escherichia coli* on peptone-sucrose agar. *A*, No acid added; no growth of *Dictyostelium*, the spores remaining ungerminated 10 days after inoculation. *B*, 0.5 cc sterile N/2 HCl added during 3-day period. Bacterial colonies nearest the point of acid application have been consumed by the *Dictyostelium*, those somewhat more removed have been partially consumed, while those farthest removed remain intact; thus the growth of the slime mold can be correlated with the outward diffusion of the added acid. *C*, Same culture as *B*, but 7 days older. All bacterial colonies have been consumed by the *Dictyostelium*, but sorocarps have developed only in the four nearest the point of acid application.

ion concentration apart from any carbohydrate fermentation could be determined.

Plates of an agar medium containing 1.2 percent each of peptone and sucrose were inoculated with *Escherichia coli* in the manner shown in figure 6, *A;* and 3 days later the resulting bacterial colonies were inoculated with pure spores of *Dictyostelium discoideum*. Immediately thereafter 2 drops of steam-sterilized N/2 hydrochloric acid were added to duplicate cultures, as shown in figure 6, *B*, and additional acid up to approximately 0.5 cc was added over a period of 3 days, 1 or 2 drops being added at 12-hour intervals. The results of this preliminary experiment are diagrammatically shown in figure 6. *Dictyostelium discoideum* failed to grow in cultures to which no acid was added; but

it did grow in cultures to which acid was added, and greater growth and more nearly normal development took place in the bacterial colonies closest to the points of acid application.

Since *Dictyostelium* failed to grow in control cultures to which no acid was added, its growth in acidified cultures could be attributed solely to the favorable influence of the added acid. On the other hand, since *Dictyostelium* grew best in bacterial colonies nearest the sites of acid application but even there was abnormal in its development (fig. 6, *C*), it seemed probable that the quantity of acid used was insufficient to counteract completely the alkaline reaction of the bacterial colonies. Accordingly, in the succeeding experiment increased quantities of sterile acid were employed.

For this succeeding investigation, as for the preliminary experiment just considered, agar media containing 1.2 percent each of peptone and sucrose were employed; and, as in the earlier study with added fungi, the indicator bromocresol purple was included in the medium in order that the changes in the hydrogen-ion concentration of the cultures could be more easily followed.

Escherichia coli and *Pseudomonas fluorescens* were again selected as bacterial associates. Neither species can ferment sucrose, and from earlier studies it was known that upon peptone-sucrose media these bacteria produced colonies in which *Dictyostelium* could not ordinarily grow. Incubation of the cultures was at 20° to 23° C. throughout the experiment.

To understand better the influence of the acid, the quantitative relation between the medium and the added acid was carefully considered. In all cases Petri plates containing 20 cc of medium were used, and different amounts of acid were subsequently added. Eight plates were inoculated with *Escherichia coli* and a like number with *Pseudomonas fluorescens*. At the end of 3 days the resulting colonies of bacteria were inoculated with pure spores of *Dictyostelium discoideum*, and immediately thereafter the addition of acid was begun. Of each series of eight plates two were kept as controls and received no acid, two received 0.5 cc, two 1.0 cc, and two 1.5 cc of steam-sterilized N/2 hydrochloric acid. In each case the acid was added in five equal applications, the first being made at the time the spores were introduced and the three successive applications being made at 12-hour intervals thereafter. The fifth and final application was delayed in all cases until the fourth day after spore introduction, and was then added primarily to maintain the reaction then obtaining in the cultures rather than to render them more acid. In all cases the acid was added by means of sterile 1-cc pipettes graduated in 0.01-cc units.

A record of the growth of *Dictyostelium discoideum* in the different cultures was made on the eighth day after the introduction of the slime mold and is presented in table 3, while a graphic comparison of the amount of growth under the different culture conditions is shown in figure 7.

Dictyostelium discoideum failed to grow in colonies of *Escherichia coli* or *Pseudomonas fluorescens* in the absence of added acid; while, within the limits of the experiment, the growth of *Dictyostelium* increased progressively as the quantity of added acid was augmented (fig. 7, *A*, *B*). In this connection, however, it should be noted that in cultures to which 1.5 cc of sterile N/2 hydrochloric acid was added portions of the bacterial colonies nearest the sites of acid application

were rendered very acid, and in these strongly acid zones *D. discoideum* failed to grow (fig. 8, *B*, *C*).

In another experiment paralleling that described above, similar quantities of acid were added to cultures grown upon media containing

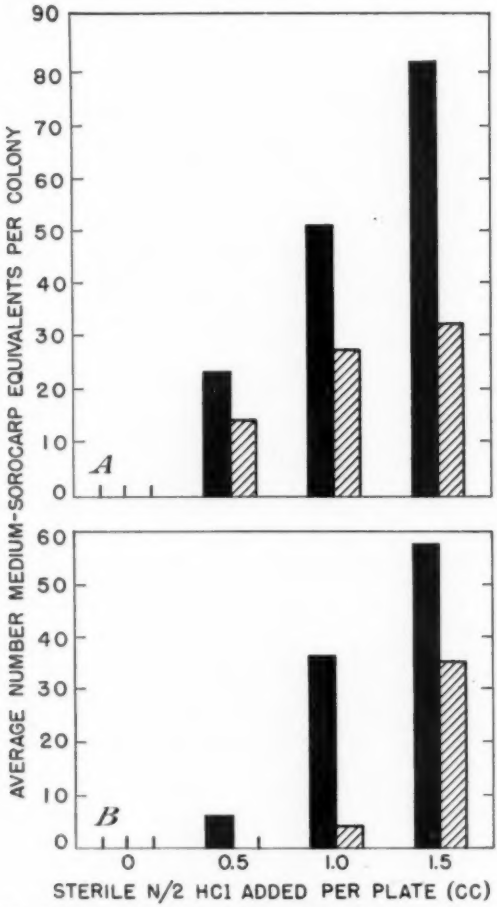


FIGURE 7.—Comparative growth of *Dictyostelium discoideum* in association with *Escherichia coli (A)* and with *Pseudomonas fluorescens (B)* upon peptone-sucrose agar plates to which different quantities of sterile N/2 HCl were added. Solid columns represent colonies adjacent to added acid; broken columns, colonies farther removed from added acid.

peptone alone, with results essentially duplicating those observed in cultures on peptone-sucrose media.

Particular quantities of acid permitted a greater growth of *Dictyostelium* when added to cultures of *Escherichia coli* than when added to cultures of *Pseudomonas fluorescens* (fig. 7). This, however, was to

be expected, since *Ps. fluorescens* is the more active proteolytic organism of the two and therefore produces more ammonia than *E. coli* on media containing peptone.

In general the pseudoplasmodia migrated toward the acid portions of the culture plates. This response is well illustrated in figure 8,

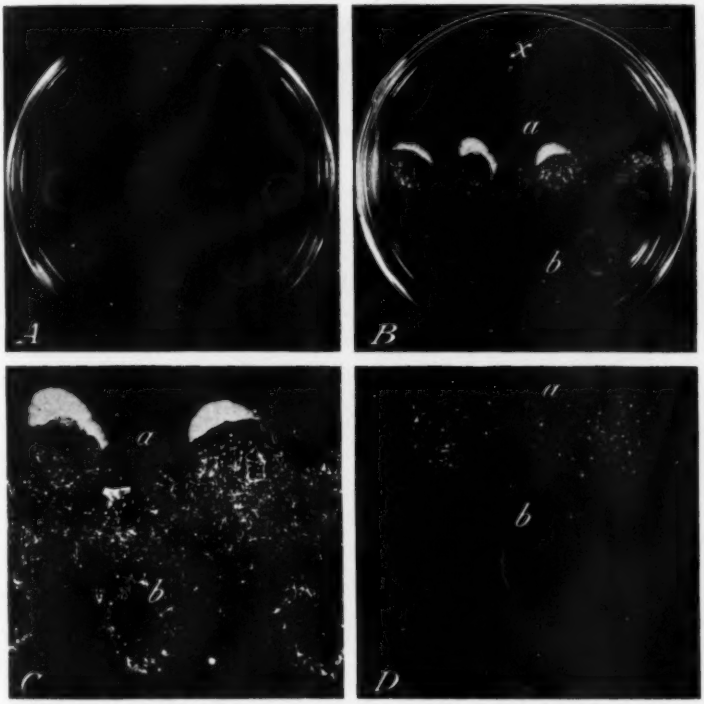


FIGURE 8.—Influence of added acid upon the growth and development of *Dictyostelium discoideum* in association with *Escherichia coli* on peptone-rich agar media containing the indicator bromocresol purple. *A*, Peptone-sucrose medium; no acid added; no growth of *Dictyostelium;* 10 days after inoculation; × ½. *B*, Peptone-sucrose medium; acidified culture, 1.5 cc of sterile N/2 HCl added at *x* during 3-day period following inoculation with *Dictyostelium;* four bacterial colonies in acid zone (*a*) largely consumed (crescent-shaped uncleared zones too acid for *Dictyostelium*) and abundant sorocarps present; colonies in the neutral or alkaline zone (*b*) partially consumed, many pseudoplasmodia present; 5 days; × ½. *C*, Central area of *B;* strongly acid portions (*a*) of bacterial colonies still untouched, remainder (*b*) completely consumed by the slime mold; 10 days; × 1⅛. *D*, Peptone medium; acidified culture, 1.0 cc of sterile N/2 HCl added as in *B;* concentration of sorocarps along the border line between the acid zone (*a*) and the neutral or alkaline zone (*b*) of the culture plate indicates a positive chemotactic response to acid by the migrating pseudoplasmodia; 10 days after inoculation; × 1¼. (Photographs by Frank White.)

C and *D*, where the sorocarps are seen clustered at the junction of the acid and alkaline zones in the medium. Since the cultures were grown in continuous darkness, this can apparently be regarded as a true chemotactic response.

TABLE 3.—*Growth of Dictyostelium discoideum in colonies of Escherichia coli and Pseudomonas fluorescens on peptone-sucrose medium in the presence of different quantities of added sterile N/2 hydrochloric acid*

Bacterial associate	Quantity of N/2 HCl added per plate	Area of plates rendered acid	Growth of *Dictyostelium discoideum*											
			Average growth per colony in row adjacent to site of acid application						Average growth per colony in row removed from site of acid application					
			Extent of colony clearance	Sorocarps				Typical sorocarps	Extent of colony clearance	Sorocarps				Typical sorocarps
				Large	Medium	Small	Medium-sorocarp equivalents			Large	Medium	Small	Medium-sorocarp equivalents	
	Cc	*Percent*		*Number*	*Number*	*Number*	*Number*	*Percent*		*Number*	*Number*	*Number*	*Number*	*Percent*
Escherichia coli	0	0	None	0	0	0	0	0	None	0	0	0	0	0
	.5	0	Complete	3–4	8–10	8–10	23	20	Complete	2–3	4–6	3–4	14	0
	1.0	35	do	8–10	15–20	10–15	51	65	do	4–5	8–10	10–12	27	20
	1.5	65	Largely complete [1]	12–15	20–25	10–15	82	75	do	5–6	10–12	12–15	32	40
Pseudomonas fluorescens	0	0	None	0	0	0	0	0	None	0	0	0	0	0
	.5	0	Slight	0–1	3–4	4–5	6	0	do	0	0	0	0	0
	1.0	25	Partial	6–8	10–12	4–6	37	70	Slight	0	[2] 3–4	0	4	0
	1.5	75	do.[1]	10–12	15–20	8–10	58	50	Complete	6–8	8–10	8–10	35	40

[1] In colonies nearest the sites of acid application crescent-shaped zones of bacterial colonies became very acid in reaction and were not consumed by the feeding myxamoebae (fig. 8, *B*, *C*).

[2] Irregular masses of myxamoebae; no true sorocarps formed.

Greater growth of the slime mold occurred in the bacterial colonies adjacent to the points of acid application than in those more removed from these sites; and in all cases a greater percentage of the sorocarps developing near the boundary of the acid zone was of normal pattern than of those developing elsewhere (fig. 8, *C*, *D*). Together these facts indicated that a reaction somewhere between pH 7.0 and 5.5 was optimum for *Dictyostelium discoideum*. Further, it was shown that while a weak acid reaction favored the growth of *D. discoideum* a strong acid reaction, like an alkaline one, inhibited its growth and development.

USE OF BUFFERED AND ADJUSTED MEDIA

It was now evident that bacterial colonies of a mild acid reaction especially favored *Dictyostelium discoideum*. However, neither the range of pH within which the slime mold could grow nor the optimum pH for its growth and development was yet definitely known. In the succeeding experiments an investigation of these questions was undertaken through the use of buffered media which were adjusted to different initial reactions by the addition of N/1 hydrochloric acid or N/1 sodium hydroxide.

For the first of these experiments an agar medium containing 1.2 percent peptone was employed. A part of this medium was reserved as a control and was neither buffered nor adjusted, while the remainder was buffered with equimolar quantities of monobasic potassium phosphate and dibasic sodium phosphate and was subsequently adjusted to different pH values by the addition of N/1 hydrochloric acid. Altogether five media were prepared which were constant in nutrient but which differed one from another either in the amount of buffer they contained or in their pH adjustment (table 4).

Again *Escherichia coli* and *Pseudomonas fluorescens* were selected as bacterial associates. Since it was known that neither of these species produced colonies favoring *Dictyostelium* when grown upon peptone-rich media in the absence of buffers and pH adjustments, any growth of the slime mold that might occur upon buffered and adjusted media of like nutrient composition could be attributed to the action of the buffers in maintaining a proper pH adjustment.

Duplicate plates of the five media were inoculated with *Escherichia coli* and a like number with *Pseudomonas fluorescens* in the manner shown in figure 9. The resulting bacterial colonies were seeded with pure spores of *Dictyostelium* when 2½ days old. Incubation throughout the experiment was at room temperature (20° to 24° C.).

Dictyostelium discoideum failed to grow in cultures of either *Escherichia coli* or *Pseudomonas fluorescens* on the unbuffered and unadjusted media where the bacterial colonies became markedly alkaline in reaction; whereas it thrived in cultures of these bacteria upon buffered and adjusted media of the same nutrient content where the bacterial colonies remained acid in reaction (table 4). Thus the experiment further substantiated the writer's earlier observations that an acid reaction favored the slime mold. In addition, it clearly demonstrated that the amount of growth and the character of development of *Dictyostelium* could be in some measure correlated with the degree of acidity existing within the bacterial colonies into which it was introduced (table 4). For example, it failed to grow in bacterial colonies more alkaline than pH 8.0; grew poorly or fairly and developed abnormally in colonies of pH 7.6 to 8.0; grew well in colonies of pH 6.8

TABLE 4.—*Influence of buffer concentrations and of pH adjustment upon the growth of Dictyostelium discoideum in colonies of Escherichia coli and Pseudomonas fluorescens on media containing 1.2 percent of peptone as the sole nutrient*

Bacterial associate	Buffer used in peptone medium	Initial pH [1]	Growth of bacteria [2]	Growth of *Dictyostelium discoideum*							Final pH [4]
				Clearance of bacterial colonies	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Migration of pseudoplasmodia [3]	
						Large	Medium	Small	Medium sorocarp equivalents			
						Number	*Number*	*Number*	*Number*	*Percent*		
Escherichia coli	None	6.8–7.0	*C*	None	None	0	0	0	0	0	None	8.0–8.2
	M/150 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$.	6.0–6.2	*C*	Partial	Poor	1–3	1–3	0	9	0	Limited, up to 1 cm	7.8–8.0
	do	5.2–5.4	*C*	Nearly complete	Fair	3–5	4–5	3–4	19	25	do	7.6–7.8
	M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$.	6.0–6.2	*C*	Complete	Good	25–30	30–40	15–20	134	90	Extensive, up to 3 cm.	6.8–7.0
	do	5.2–5.4	*C*	do	Very good	30–35	40–50	20–25	145	90	do	6.4–6.6
Pseudomonas fluorescens.	None	6.8–7.0	*C*	None	None	0	0	0	0	0	None	8.4–8.6
	M/150 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$.	6.0–6.2	*C+*	do	do	0	0	0	0	0	do	8.2–8.4
	do	5.2–5.4	*C+*	Partial	Poor	0	0–2	0	1	0	do	8.0–8.2
	M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$.	6.0–6.2	*C*	Complete	Fair	4–6	8–10	10–15	28	70	do	7.0–7.2
	do	5.2–5.4	*C*	do	Good	12–15	25–30	15–20	78	85	Extensive, up to 2.5 cm.	6.8–7.0

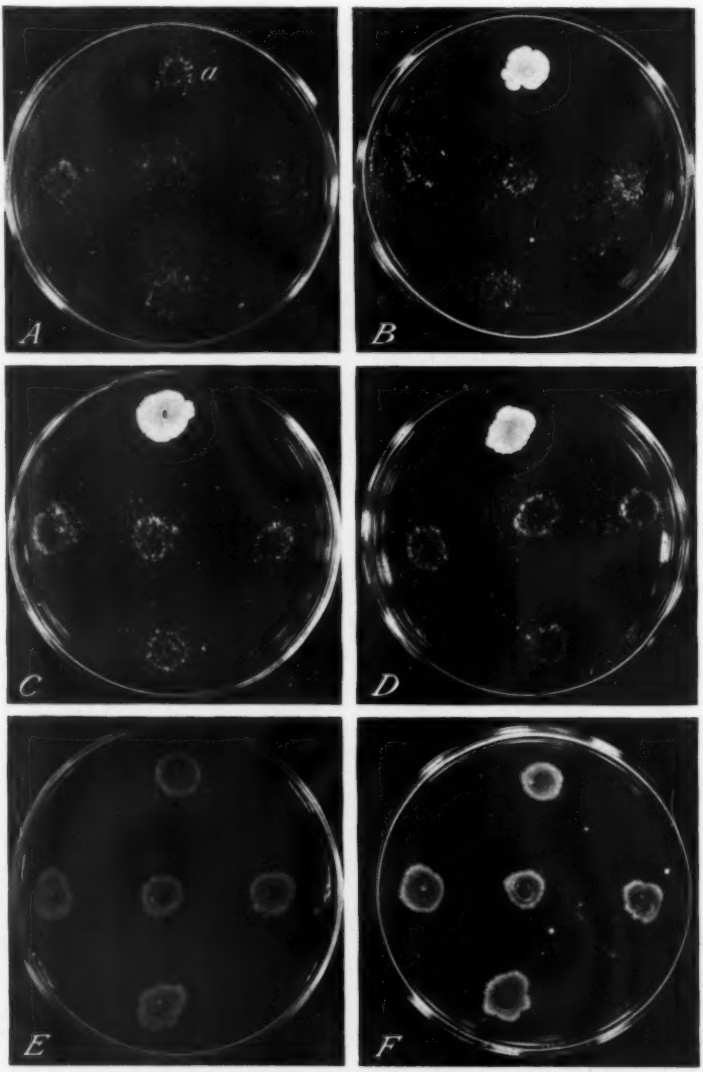
[1] The pH of the medium at the time the bacteria were inoculated.
[2] Growth is designated by the symbol *C* for a standard growth of *E. coli* upon a medium containing 12 g of lactose and 4 g of peptone per liter (p. 165).
[3] Migration of pseudoplasmodia is recorded in the maximum distance any pseudoplasmodium migrated from the bacterial colony in which it formed.
[4] The pH of the medium adjacent to the colonies of bacteria or bacteria and *Dictyostelium* at the end of the experiment.

TABLE 5.—*Influence of hydrogen-ion concentration upon the growth of Dictyostelium discoideum in colonies of Escherichia coli and of Pseudomonas fluorescens growing upon 1.2 percent peptone agar media which was also M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$*

Bacterial associate	pH adjustment	Bacterial growth [1]	Growth of *Dictyostelium discoideum*							Change of pH		Migration of pseudoplasmodia [4]
			Clearance of bacterial colony	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Initial [2]	Final [3]	
					Large	Medium	Small	Medium-sorocarp equivalents				
					Number	*Number*	*Number*	*Number*	*Percent*			
Escherichia coli	3.0±	Negligible	None	None	0	0	0	0	0	3.0–3.2	3.2–3.4	None.
	4.0±	*C*	Complete	Excellent	30–35	40–50	30–40	165	70	4.0–4.2	5.6–5.8	Very extensive, up to 3.5 cm.
	5.0±	*C*	do	do	30–35	30–35	25–30	151	85	5.0–5.2	6.2–6.4	Extensive, up to 3 cm.
	6.0±	*C*	do	Very good	20–25	20–30	20–30	108	75	6.0–6.2	6.6–6.8	Limited, up to 1.5 cm.
	7.0±	*C*	Nearly complete	Good	10–12	15–20	10–15	58	50	6.8–7.0	7.2–7.4	Limited, up to 1.0 cm.
	8.0±	*C*	Slight	Very poor	0	0	0	0	0	8.0–8.2	8.4–8.6	None.
	9.0±	*C*	None	None	0	0	0	0	0	8.8–9.0	9.0–9.2	Do.
	[5]	*C*	Slight	Very poor	0	0	0	0	0	6.8–7.0	8.2–8.4	Do.
Pseudomonas fluorescens.	3.0±	None	None	None	0	0	0	0	0	3.0–3.2	3.0–3.2	Do.
	4.0±	*C*	Complete	Excellent	35–40	40–50	30–40	182	75	4.0–4.2	6.0–6.2	Very extensive, up to 4 cm.
	5.0±	*C*	do	do	30–35	30–40	20–25	153	70	5.0–5.2	6.8–7.0	Extensive, up to 2.5 cm.
	6.0±	*C*+	Partial	Poor	0–2	0–4	0–4	6	0	6.0–6.2	7.6–7.8	None.
	7.0±	*C*+	Slight	Very poor	0	0–3	0–4	2	0	6.8–7.0	8.2–8.4	Do.
	8.0±	*C*+	None	None	0	0	0	0	0	8.0–8.2	8.6–8.8	Do.
	9.0±	*C*+	do	do	0	0	0	0	0	8.8–9.0	9.2–9.4	Do.
	[5]	*C*+	do	do	0	0	0	0	0	6.8–7.0	8.6–8.8	Do.

[1] Growth of bacteria is expressed in terms of a standard growth of *E. coli* (*C*) upon a medium containing 12 g of lactose and 4 g of peptone per liter.
[2] Determinations made at time bacteria were inoculated upon media.
[3] Determinations made at close of experiment; readings taken from zones adjacent to colonies.
[4] Recorded in maximum distance any pseudoplasmodium traveled from site of formation.
[5] Unbuffered; unadjusted.

to 7.0; and grew luxuriantly and developed in a wholly normal manner in colonies of pH 6.4 to 6.6. In addition to the correlation between the degree of acidity and the total growth of the slime mold, there also appeared to be a positive correlation between the amount of growth and the extent of pseudoplasmodial migration, and similarly between the quantity of growth and the percentage of normal sorocarps developed.



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The growth of *Dictyostelium* was uniformly better in cultures of *Escherichia coli* than in those of *Pseudomonas fluorescens* upon similar media, a result which was not unexpected since *Ps. fluorescens* is more actively proteolytic than *E. coli*.

A buffer concentration of M/150 in monobasic potassium phosphate (KH_2PO_4) and of M/150 in dibasic sodium phosphate ($Na_2HPO_4+12H_2O$) proved inadequate to maintain a favorable acid reaction in colonies of either bacterial associate upon the peptone medium employed. However, a concentration of M/50 in each of these phosphates was sufficient to maintain a mild acid reaction in colonies of the bacteria, and in such cultures *Dictyostelium discoideum* grew luxuriantly and developed normally.

Using the stronger buffer concentration (i. e., M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$), a second and more extensive study was now undertaken for the dual purpose of further testing the observations already made and of determining whether or not a lower pH adjustment than those previously studied would be even more favorable for *Dictyostelium discoideum*. For this investigation an agar medium containing 1.2 percent of peptone and the buffer concentration indicated above was prepared. Portions of this stock medium were then adjusted to reactions of approximately pH 3.0,[11] 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 by the addition of N/1 hydrochloric acid or N/1 sodium hydroxide.

Duplicate plates of each medium, including an unbuffered and unadjusted one, were inoculated with *Escherichia coli* and a similar number with *Pseudomonas fluorescens*. As in the preceding experiments, these cultures were incubated at room temperature (22°–24° C.) and the resultant colonies were seeded with pure spores of *Dictyostelium* after 3 days.

A record of the growth and character of development of the slime mold in the several cultures is presented in table 5, while a graphic comparison of its growth in the different cultures is shown in figure 10.

Both bacterial associates tended strongly to shift the pH of the cultures toward an alkaline reaction (table 5) owing to the liberation of ammonia, and it is essential to recognize that the initial pH of any

[11] To obtain a firm agar gel in strong acid media, additional agar was added up to 3 percent for the media of pH 3.0 and 2.5 percent for the media of pH 4.0; pH adjustments were made by the addition of sterile N/1 HCl after the agar media were sterilized.

EXPLANATORY LEGEND FOR FIGURE 9

FIGURE 9.—Ten-day-old cultures of *Dictyostelium discoideum* in colonies of *Escherichia coli* on buffered peptone-agar media initially adjusted to different hydrogen-ion concentrations. × ½. *A*, Initial pH 4.0±; luxuriant growth, scattered sorocarps indicate extensive migration of pseudoplasmodia; uninoculated colony (*a*) invaded by scattered myxamoebae and now indistinguishable from colonies originally inoculated; final reaction adjacent to colonies, pH 5.6–5.8. *B*, Initial pH 5.0±; slightly reduced growth and less extensive migration of pseudoplasmodia than in preceding culture; final reaction adjacent to colonies, pH 6.2–6.4. *C*, Initial pH 6.0± further reduction in the amount of growth and in the extent of pseudoplasmodial migration; final reaction adjacent to colonies, pH 6.6–6.8. *D*, Initial pH 7.0±; still further reduction in growth and in the migration of pseudoplasmodia; final reaction adjacent to colonies, pH 7.2–7.4. *E*, Initial pH 8.0±; small areas of bacterial colonies consumed by myxamoebae but no pseudoplasmodia produced; final reaction adjacent to colonies, pH 8.4–8.6. *F*, Initial pH 9.0±; no growth of *Dictyostelium*, the spores remaining ungerminated; final reaction adjacent to colonies, pH 9.0–9.2. (Photographs by Frank White.)

medium was of importance only insofar as it influenced the growth of the host bacteria and the pH of the bacterial colonies during the growth period of the slime mold. Determinations of the pH of bacterial colonies were not made at the time of inoculation with *Dictyostelium*, but since the bacteria had almost if not wholly ceased growing by that time, it is believed that the determinations made at the close of the experiment fairly represent the pH of the bacterial colonies throughout the whole period of slime mold growth.

The growth of *Dictyostelium discoideum* could be closely correlated with the hydrogen-ion concentration of the host bacterial colonies (table 5). Spores of *Dictyostelium* failed to germinate in colonies with a final reaction in excess of pH 8.5, and myxamoebae grew very poorly or not at all in colonies with a reaction of pH 8.0 or above. Poor growth of myxamoebae occurred and abnormal sorocarps were developed, but no migration of pseudoplasmodia took place from colonies with a final reaction between pH 7.5 and 8.0; fair to good growth of *Dictyostelium* occurred in colonies of pH 7.0 to 7.4, but a large per-

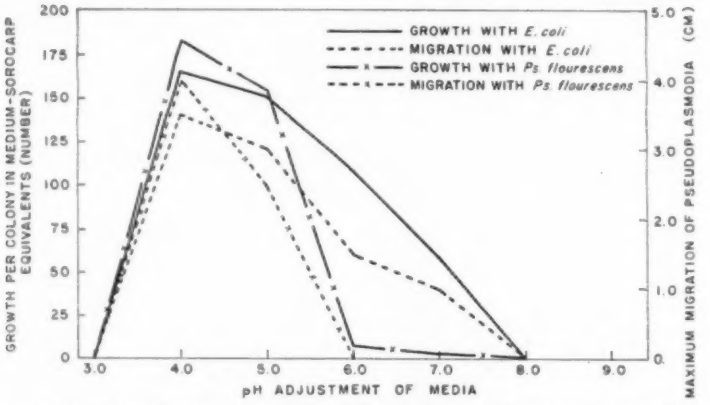

FIGURE 10.—Comparative growth and pseudoplasmodial migration of *Dictyostelium discoideum* in association with *Escherichia coli* and with *Pseudomonas fluorescens* upon buffered peptone-agar media of different initial pH adjustments.

centage of the sorocarps produced were of abnormal pattern; whereas very good to excellent growth and wholly normal development of *D. discoideum* occurred in colonies of bacteria in which the final reaction was between pH 5.0 and 7.0. The optimum reaction appeared to be in the neighborhood of pH 6.0.

Neither *Escherichia coli* nor *Pseudomonas fluorescens* grew upon media adjusted to pH 3.0; and since there were no bacteria present *Dictyostelium discoideum* could not grow for lack of food, quite aside from any effect the strong acid reaction in itself might have had upon its growth and development.

The percentage of typical sorocarps could in general be correlated with the amount of growth (table 5). That is, a bacterial colony that favored a luxuriant growth of *Dictyostelium* also favored the production of a large percentage of normal sorocarps. Also the ratio of normal to abnormal sorocarps was regularly much greater among soro-

carps resulting from migrating pseudoplasmodia than among sorocarps produced from nonmigrating pseudoplasmodia. This was to be expected, for it could be assumed that the more vigorous pseudoplasmodia would migrate and that they also would produce sorocarps of the pattern most characteristic of the species. The extent of migration of pseudoplasmodia could likewise be correlated with the favorableness of the culture. That is, where the greatest growth and most nearly normal development of *Dictyostelium* occurred, there also occurred the greatest migration of pseudoplasmodia, not only in the number migrating but also in the maximum distances traveled by individual pseudoplasmodia (fig. 10).

Paralleling the study described above, a similar experiment was conducted in which a stock medium containing 1.2 percent each of peptone and sucrose was substituted for the one containing peptone alone. This yielded results essentially like those obtained with the peptone media except that with both *Escherichia coli* and *Pseudomonas fluorescens* maximum growth of *Dictyostelium discoideum* occurred upon media initially adjusted to pH 5.0 instead of 4.0. As with the cultures upon peptone media, however, maximum growth of the slime mold occurred in bacterial colonies with a final reaction of approximately pH 6.0, while the percentage of normal sorocarps and the extent of pseudoplasmodial migration could again be correlated with the total growth of the slime mold.

Thus it was demonstrated that the growth of *Dictyostelium discoideum* is governed by the hydrogen-ion concentration of the bacterial colonies into which it is introduced, and that a reaction of pH 6.0± is apparently optimum for its growth and normal development. Furthermore, it was shown that such characters of the fruiting stage as pseudoplasmodial migration and sorocarp pattern can be used as an index to the favorableness of a culture.

INCUBATION TEMPERATURE OF BACTERIA PRIOR TO THE INTRODUCTION OF DICTYOSTELIUM

During the course of previous investigations it was occasionally, though rarely, noted that *Dictyostelium discoideum* grew poorly and irregularly in colonies of *Escherichia coli* upon media containing 1.2 percent each of peptone and lactose. This was indeed puzzling because excellent growth normally occurred in colonies of this bacterium upon media of the above-mentioned composition. A possible clue was obtained when the pH of such cultures was found to be uniformly of a strong acid reaction, ranging from pH 4.0 to 4.5. Certain possible explanations of this phenomenon presented themselves; but the temperature at which the bacterial cultures were incubated prior to inoculation with *Dictyostelium* offered the most promising lead, for it was observed that in all cases such cultures had been exposed for longer or shorter periods to temperatures somewhat in excess of the normal temperature of the laboratory.

In order to investigate this lead, therefore, plates of agar media containing 1.2 percent each of peptone and lactose were inoculated with *Escherichia coli* and incubated for 3 days, some at 35° C., some at 25° to 26°, and others at 15°. Pure spores of *Dictyostelium* were introduced into the bacterial colonies after 3 days, and all of the cultures were subsequently incubated at the uniform temperature of 22° to 23°.

TABLE 6.—*Growth of Dictyostelium discoideum in colonies of Escherichia coli grown upon lactose-peptone media at different temperatures for 2 days prior to the introduction of Dictyostelium discoideum spores*

Incubation temperature of bacteria (° C.)	Bacterial growth [1]	Growth of *Dictyostelium discoideum*								pH		
		Clearance of bacterial colony	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Migration of pseudoplasmodia	Initial pH of medium	Final pH [2] in colonies	Final pH between colonies
				Large	Medium	Small	Medium-sorocarp equivalents per colony					
				Number	*Number*	*Number*	*Number*	*Percent*				
35°	*C*	Partial	Poor	0	0	0	0	0	None	6.8–7.0	4.0–4.2	4.4–4.6
25°–26°	*C*+	Nearly complete	Good	8–10	10–15	6–10	45	55	Up to 2.5 cm	6.8–7.0	4.4–4.6	5.3–5.5
15°	*C*	Complete	Very good	25–30	25–30	20–30	130	75	Up to 4.5 cm	6.8–7.0	6.6–6.8	6.4–6.6

[1] Growth is expressed in terms of a standard growth of *E. coli* (*C*) upon a medium containing 12 g of lactose and 4 g of peptone per liter.
[2] Determinations made upon agar surface immediately adjacent to the bacterial colonies.

The results of the experiment, which are shown in table 6, are self-explanatory, but the following points are worthy of special attention: (1) The final reaction of bacterial colonies in cultures initially incubated at 35° C. was strongly acid, pH 4.0 to 4.2, and the growth of *Dictyostelium* was extremely poor and its development very abnormal. (2) The final reaction of colonies initially incubated at 25° to 26° was slightly less acid, pH 4.4 to 4.6, and although fair growth of the slime mold occurred, its development was abnormal in character. (3) The final reaction of bacterial colonies initially incubated at 15° was near neutral, pH 6.6 to 6.8, and the growth of *Dictyostelium* was very good and its development was essentially normal.

A similar and even more striking experiment confirming the above results was readily performed, Endo's differential medium being used. Colonies of *Escherichia coli* upon this medium when incubated at 35° C. became deep red in color, flat, dryish, and were characterized by a metallic sheen. The underlying medium also became a deep red, indicating a strong acid reaction. In contrast, colonies incubated at a temperature of 22° to 23°, or less, remained light pink in color, raised, moist, and devoid of sheen, and the underlying medium became light red, indicating a mild acid reaction. In the latter and weakly acid colonies *Dictyostelium discoideum* grew luxuriantly and developed normally, whereas it failed to grow in the former strongly acid cultures.

Thus in these experiments, as in previous ones, the growth and development of *Dictyostelium discoideum* were governed by the hydrogen-ion concentration of the bacterial colonies into which it was introduced. However, in these latter cases, the inhibition of growth and the production of abnormal fruiting structures were due to a too acid rather than a too alkaline reaction.

USE OF NITROGEN SOURCES OTHER THAN PEPTONE

Peptone was employed almost exclusively as a source of nitrogen in the present studies. However, this nutrient is not essential for the successful cultivation of *Dictyostelium discoideum* on artificial media, and other nitrogen sources, including asparagine, ammonium chloride, ammonium nitrate, and ammonium phosphate, were successfully employed in conjunction with fermentable carbohydrates. In cultures with these nitrogen sources, as in those with peptone, a definite relationship existed between the hydrogen-ion concentration of the bacterial colonies and the subsequent growth and development of *D. discoideum*. The slime mold grew poorly or not at all in bacterial colonies more alkaline than pH 8.0; whereas in colonies of pH 4.8 to 7.0 sorocarp development was for the most part normal, indicating that conditions were essentially favorable for *D. discoideum*.

STUDIES WITH NATURAL MEDIA

Following the completion of the studies with peptone-sugar media, further experimentation with natural substrata supplied additional proof of the importance of the hydrogen-ion concentration of the host bacterial colonies. In connection with preliminary studies (p. 160) it was noted that carrot-infusion agar afforded a reasonably satisfactory medium for the cultivation of *Dictyostelium discoideum* in association with *Pseudomonas fluorescens* or *Serratia marcescens;* whereas yeast

and bean-infusion agar, although supporting an ample growth of bacteria, did not permit *D. discoideum* to grow and develop normally. Therefore, it is of interest to note that the carrot-infusion agar was mildly acid in reaction at the time of inoculation with bacteria and remained so throughout the growth period of the bacteria and subsequently of the slime mold; while, on the contrary, the yeast and bean-infusion agars, although near neutral in reaction when inoculated with bacteria, became quite alkaline during the growth of the bacteria, and the growth of the slime mold was subsequently inhibited.

Pinoy (*16*) reported that *Dictyostelium mucoroides* in association with *Bacillus fluorescens liquefaciens* thrived upon pieces of sterilized carrot but could not grow with this same bacterial associate upon pieces of sterilized potato, although abundant bacteria were present for the slime mold to feed upon. Skupienski (*24*) later confirmed Pinoy's results and attributed the difference in behavior to the presence in the one medium of some essential substance that was absent from the other. The writer, however, believes that the presence of growth upon carrot and its absence upon potato were due to a difference in the hydrogen-ion concentration of the bacterial colonies upon the two substrata. As already noted, carrots and carrot agar are of a mild acid reaction and the bacterial colonies developing upon these media remain acid in reaction, and in them *D. discoideum* grows well and develops in a normal manner. On the other hand, although sterilized potato and potato-infusion agar are initially of a weak acid reaction, upon these media colonies of *Pseudomonas fluorescens* (and *Serratia marcescens* likewise) become markedly alkaline in reaction (pH 8.0–8.2 ±) and *D. discoideum* fails to grow in them. Thus it would seem that the presence of good growth of *Dictyostelium* upon sterilized carrot and its absence upon sterilized potato can be attributed to the difference in hydrogen-ion concentration of the bacterial colonies upon the two substrata.

It is of interest to call attention to the striking similarity of results obtained by the writer with *Dictyostelium discoideum* in association with *Serratia marcescens* and by Cutler and Crump with a soil amoeba, *Hartmanella hyalina* Dangeard, in association with the same bacterial species. Cutler and Crump in 1927 (*5*) reported that *H. hyalina* could not feed upon *Bacillus prodigiosus* Flügge (*S. marcescens*), but in 1935 (*6*) they observed that the amoeba could grow with this bacterium in soil extract and pointed out that in their earlier work they had attempted to grow the two organisms upon nutrient agar. In the present study the writer found that *D. discoideum* could not grow with *S. marcescens* upon nutrient agar but that it grew well with this bacterium upon hay-infusion agar (*19*), a medium more or less comparable in nutritive value to the soil extract employed by Cutler and Crump. Upon nutrient agar the bacterial colonies became strongly alkaline, while upon hay-infusion agar they remained slightly acid or became neutral in reaction.

The writer is not prepared to say what role the hydrogen-ion concentration of substrata and of host bacterial colonies plays in governing the growth of soil amoebae generally, but the case at hand is suggestive and is of particular interest because of the remarkable parallelism of behavior shown.

DISCUSSION

In this study the formation or nonformation of sorocarps has been considered the primary measure of the favorableness or unfavorableness of cultures to *Dictyostelium discoideum*, while the form or pattern of the sorocarps produced has been interpreted as indicating the degree of favorableness of cultures. This practice is based upon consistent results obtained by the writer from repeated experiments, and although it was not employed by earlier students of the Dictyosteliaceae, it is wholly reliable and affords a most convenient means of evaluating the worth of different combinations of culture media and bacterial associates.

In *Dictyostelium discoideum*, as in all the Dictyosteliaceae, the vegetative phase and the fruiting phase are clearly set apart; and from the results of the current studies it is plainly evident that these two phenomena are not influenced, retarded, or inhibited to the same extent by the presence of toxic conditions within the colonies of host bacteria. Degrees of inhibition can be readily distinguished and these can be correlated with the nutrient content of the media and the fermentations of the associated bacteria (tables 1 and 2), and likewise with the hydrogen-ion concentration of the host bacterial colonies (tables 3, 4, 5, and 6). In bacterial colonies more alkaline than pH 8.6, the spores of *D. discoideum* fail to germinate; in colonies of pH 8.2 to 8.6, limited myxamoebic growth normally occurs and an occasional pseudoplasmodium forms but no sorocarps develop; in colonies of pH 7.5 to 8.0, fair growth of the slime mold ordinarily takes place, limited numbers of pseudoplasmodia form, and some sorocarps develop but the majority of these are of abnormal pattern (fig. 3, *E–I*); while in colonies of pH 5.0 to 7.0, excellent growth of *Dictyostelium* invariably occurs, numerous pseudoplasmodia form, and the great majority of these subsequently develop into sorocarps of normal form and proportions (fig. 3, *A–C*).

In colonies more acid than pH 5.0 there is likewise a steady reduction in the amount of growth accompanied by an increasingly abnormal development of the slime mold as the acidity increases. However, the limiting influence of acidity has not been adequately investigated as yet, and reactions at which different degrees of inhibition occur cannot be given.

Further consideration of the significance of sorocarp patterns is believed worth while. The process of sorocarp formation is delicately sensitive to culture conditions, and slight differences in environment are rendered apparent during the formation of fruiting structures. Under optimum culture conditions, the host bacterial colonies are completely consumed and all of the myxamoebae, with the exception of scattered individuals, aggregate into pseudoplasmodia which subsequently resolve into sorocarps of normal pattern that are entirely typical for the species; whereas, under less favorable conditions the bacterial colonies remain incompletely consumed, a large proportion of the myxamoebae never aggregate into pseudoplasmodia, and the sorocarps formed become increasingly less normal in pattern as conditions become more unfavorable until finally they cease to be formed altogether.

The first indication of unfavorable culture conditions, as reflected in sorocarp pattern, appears in the stalk, or sorophore, and basal disk.

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The stalk is shorter, thicker, and more strongly tapered than in typical structures, while the basal disk is less expanded and more conical in shape and tends to merge imperceptibly into the base of the stalk. In such mildly abnormal sorocarps (fig. 3, *D* and *E*) the sorus appears relatively large and out of proportion to the size of the fruiting structure as a whole. As conditions become more unfavorable, the stalk becomes even shorter and thicker and the basal disk less apparent, while the sorus loses its characteristic form and appears to blend into the supporting stalk (fig. 3, *F*) in the manner of a fruiting structure of *Guttulina* (*14*). Under still less favorable conditions the myxamoebae become heaped up into a mass in which there is no differentiation either into fertile spores or characteristic stalk cells (fig. 3, *G*), the whole structure superficially resembling a fruiting mass of the simple genus *Guttulinopsis* (*14*). Finally under the least favorable conditions where growth of *D. discoideum* is possible, the myxamoebae grow meagerly and consume a limited portion of the host bacterial colony but are subsequently incapable of aggregating into pseudoplasmodia.

During the present study considerable evidence has accumulated indicating that either incubation at a temperature somewhat in excess of the optimum for this species or exposure for brief periods to the unsaturated air of the laboratory will bring about the formation of abnormal sorocarps in cultures characterized by a favorable hydrogen-ion concentration. However, in the experiments discussed herein these factors have not been of importance, since in all cases the cultures were incubated at temperatures favorable to *Dictyostelium discoideum* (18° to 24° C.), and in all cases the cultures were grown in closed Petri plates consisting of close-fitting valves that insured the maintenance of a saturated or near-saturated atmosphere within the cultures.

In the writer's experience the following factors are of primary importance in governing the growth and development of *Dictyostelium discoideum:* (1) The composition of the culture medium, (2) the fermentations of the associated bacteria, (3) the hydrogen-ion concentration of the host bacterial colonies, and (4) the physical character of the bacterial growth.

Combinations of these factors operate in any given culture to determine whether or not good growth and normal development of the slime mold can take place; and in no case is it possible to consider either the first or the second of these factors apart from the other, for it is only through one that the other becomes operative. *Dictyostelium discoideum* grows luxuriantly and develops normally at room temperature in colonies of bacteria upon media containing 1.2 percent peptone and an equal quantity of some carbohydrate that the associated bacteria can ferment; whereas it grows very poorly or not at all in colonies of the same bacteria upon media containing a like quantity of peptone as the sole nutrient, or combined with an unavailable carbohydrate (fig. 4). Clearly then, upon peptone-rich media, good growth and normal development of *D. discoideum* can be attributed to the presence of a carbohydrate that can be utilized by the associated bacteria. Peptone, or some other nitrogen-rich nutrient, cannot be omitted from such a medium, for in its absence the associated bacteria grow sparingly and the growth of the slime mold is limited accordingly. On the other hand, an available carbohydrate cannot be omitted, for, although its presence does not materially enhance the growth of host

bacteria (table 1), it insures that the slime mold can feed upon the bacteria that are present.

The importance of the nutrients used and of the fermentations by the host bacteria becomes progressively lessened as media containing successively lower concentrations of nutrients are employed. Upon media containing 1.2 percent peptone as the sole nutrient, the growth of *Dictyostelium* is very slight or wholly lacking; whereas upon media containing 0.1 percent peptone, although the growth of the slime mold is restricted in amount, owing to the limited growth of host bacteria, colonies of bacteria are wholly consumed and sorocarps of essentially normal pattern are produced (table 1).

The hydrogen-ion concentration of the host bacterial colonies is, in the writer's experience, the most important factor governing the growth and development of *Dictyostelium discoideum*. And, indeed, it is primarily through their influence upon the pH of the colonies of associated bacteria that the above factors of medium composition and bacterial fermentations operate. Optimum growth and development of *D. discoideum* occur in bacterial colonies of approximately pH 6.0, while good growth and normal development regularly occur in colonies having a reaction between pH 5.0 and 7.0. Outside of this favorable pH range, the growth of the slime mold becomes progressively less in amount and its development increasingly more abnormal as the colonies of host bacteria become either more alkaline or more acid. As demonstrated by repeated experiments, the manner in which a favorable reaction is established in the bacterial colonies is relatively unimportant; and it matters not whether it is produced by the simultaneous and balanced fermentation of some nitrogen source and some carbohydrate by the associated bacteria themselves (*1*), or by some artificial means such as the addition of free acid or the use of buffers and pH adjustments.

The results obtained from the studies with Endo's and allied media (fig. 2) and with the peptone-sugar media (table 1) can be fully explained on the basis of the hydrogen-ion concentration of the bacterial colonies, although pH determinations were not made in these early experiments. In these studies it was uniformly found that the presence or absence of good growth and normal development of *Dictyostelium discoideum* with any particular bacterial host could be correlated with the ability or inability of that bacterium to ferment the carbohydrate contained in the medium (figs. 2 and 4). And in later experiments where pH determinations were consistently made it was found that the fermentation or nonfermentation of the carbohydrate was invariably expressed in the pH of the bacterial colonies. If the carbohydrate was not fermented and only the peptone was broken down the bacterial colonies became alkaline in reaction (pH 8.4–8.6) and *Dictyostelium* failed to grow; whereas, if both the carbohydrate and the peptone were fermented the bacterial colonies became mildly acid in reaction (pH 5.5–7.0) and the slime mold grew luxuriantly and developed normally. At the same time it should be recalled that bacterial colonies of a strong acid reaction are just as toxic to *D. discoideum* as those of a marked alkaline reaction (p. 187) and when culture conditions are such that the acid products of carbohydrate fermentation outweigh the alkaline products of peptone decomposition, the bacterial colonies become strongly acid and the growth of

Dictyostelium is inhibited. Thus, if either process of decomposition overbalances the other, *Dictyostelium discoideum* is unable to grow in the resulting bacterial colonies, on the one hand because they are too alkaline in reaction (pH 8.5 and above) and on the other because they are too acid (pH 4.0 and below).

Finally, not only must the composition of the medium, the fermentations by the bacteria, and the hydrogen-ion concentration of the bacterial colonies be taken into account in order to cultivate *Dictyostelium discoideum* successfully upon peptone-sugar media, but the physical character of the bacterial growth must also be such as to favor the feeding myxamoebae. To illustrate, *Achromobacter radiobacter* (Beijerinck and Van Delden) Bergey et al., *Aerobacter aerogenes* (Kruse) Beijerinck, and *Phytomonas tumefaciens* (Smith and Townsend) Bergey et al. afford excellent nutriment for *D. discoideum* when grown upon a suitable medium such as hay-infusion agar (*19*); and among other carbohydrates each bacterium can ferment dextrose and sucrose. However, when these bacteria are grown upon media containing 1.2 percent each of peptone and dextrose or peptone and sucrose, large lens-shaped colonies of a gummy character are produced, and in these colonies the slime mold grows sparingly because of the inability of the myxamoebae to feed effectively upon the gum-encased bacterial cells. No toxic condition exists within such colonies, for the sorocarps produced, although few in number and for the most part small in size, are normal in pattern; while throughout the experiments reported in this paper it was consistently found that cultures in which toxic conditions existed, whether due to alkalinity or excess acidity, were characterized either by abnormal sorocarps or by a complete lack of fruiting structures.

Although in the writer's experience the factors just considered truly govern the growth and development of *Dictyostelium discoideum*, caution should be exercised against making too great generalizations concerning them. Different strains of the same bacterial species may vary in the physical character of their growth, while they may likewise differ appreciably in their ability to break down peptone and to ferment particular sugars; consequently, the concentrations of nutrients employed may need to be varied accordingly. Furthermore, peptones vary appreciably in character (e. g., acidity) and in nutritive value (p. 161), and such variations must also be taken into account. Thus any new combination involving either an untried strain of host bacteria or an unfamiliar medium may present new problems. However, repeated experiments have demonstrated that, so long as the hydrogen-ion concentration of the host bacterial colonies falls between pH 5.0 and 7.0 and so long as the bacterial growth is of a favorable physical character, good growth and normal development of *Dictyostelium discoideum* may be expected. Obviously, then, for the successful cultivation of this slime mold upon media rich in nutrients one should habitually employ combinations of media and bacterial associates which will yield nongummy colonies and which will enable a balanced fermentation of nutrients to take place, thereby insuring a favorable reaction in the colonies of host bacteria. Or, failing in this, one should incorporate sufficient buffers into the medium to maintain a suitable reaction regardless of the fermentative activities of the associated bacteria.

The results obtained by the writer with media containing peptone agree only in part with the findings of earlier investigators [12] who employed this nutrient. Nadson (*12*) was the first to use peptone for the cultivation of a species of *Dictyostelium* and was likewise the first to attribute a significant role to the reaction of the culture medium. He contended that the associated bacteria (*Bacillus fluorescens liquefaciens*) favored the growth of *D. mucoroides* by creating an alkaline environment. Contrary to his report for *D. mucoroides*, the writer has found that an alkaline reaction inhibits rather than favors the growth of *D. discoideum*. Olive (*14*) likewise reported successfully cultivating members of the Dictyosteliaceae upon a peptone-agar medium. However, he did not know the identity of the bacteria associated with his cultures, nor did he state the concentration of peptone employed; therefore, a close comparison between his and the writer's work cannot be made. In the event that he employed a weak concentration of peptone, the growth of the slime molds can be readily understood; for, in the writer's experience, *D. discoideum* will grow and develop more or less normally in bacterial colonies upon media containing 0.1 or 0.2 percent of peptone as the sole nutrient, although it grows poorly or not at all upon media containing higher concentrations of peptone unless this nutrient is accompanied by adequate buffers or by some carbohydrate that is fermentable by the associated bacteria.

The work by Potts (*17*) constitutes the most thorough physiological study previously made on a species of the Dictyosteliaceae. He was fully aware that, in general, sources of nitrogen yield alkaline products, whereas sources of carbon yield acid products when decomposed by bacteria. He was not unmindful of the importance of the nutrient employed, nor of the possible influence the associated bacteria might wield through altering the culture medium. Furthermore, he observed that the growth of *Dictyostelium mucoroides* was dependent on a favorable balance between these opposing products, but he did not consider the reaction of the medium as the chief factor influencing the growth of the slime mold. He reported that *D. mucoroides* grew well in cultures ranging from weakly acid to strongly alkaline, and reached its maximum growth in weakly alkaline cultures. This he determined by growing *D. mucoroides* in association with *Bacterium fimbriatum* in liquid media (presumably maize extract) containing different quantities of sodium carbonate. Contrary to the above conclusion, the results listed in tables 1 and 2 of Potts' paper show that the reaction was uniformly acid or weakly acid in cultures where the best growth of *Dictyostelium* occurred. From this conflicting evidence the writer feels justified in accepting Potts' results with many different media, as listed in his tables, in preference to the result obtained by him with a single medium in which the concentration of sodium carbonate was used as an indicator of alkalinity.

Vuillemin (*27*) successfully cultivated *Dictyostelium mucoroides* in association with *Bacillus fluorescens non-liquefaciens* upon a maltose-

[12] It should be noted that earlier investigators in all cases worked with species of *Dictyostelium* other than the one studied by the writer, and in the majority of cases with the ubiquitous species *D. mucoroides*. Nonetheless, the writer feels that in several instances a comparison of the results obtained by himself and those obtained by earlier investigators can be made, since *D. mucoroides* frequently occurs in and readily may be isolated from decaying vegetation not unlike that from which *D. discoideum* was obtained (*18*), and since the writer, in experimental studies carried on along with this work but as yet unpublished, found that conditions which favor the growth of *D. discoideum* also favor that of *D. mucoroides*.

(2.0 percent) peptone (0.5 percent) medium, but he could not grow the slime mold in association with *Bacillus pyocyaneus* Gessard upon the same medium. Knowing that *B. pyocyaneus* was an especially active ammonifier, he interpreted this result as contradicting Nadson's view (*12*) that *D. mucoroides* was favored by an alkaline reaction, a criticism which the writer's work with *D. discoideum* indicates was justified. On the other hand, since *B. fluorescens non-liquefaciens* cannot ferment maltose (*27*), the explanation of *Dictyostelium* growth with this species is not at first obvious. However, when both the weak proteolytic character of the bacterium, as evidenced by the nonliquefaction of gelatin, and the relatively low concentration of peptone are taken into account, Vuillemin's success can in large measure be explained.

Pinoy (*16*) reported that *Dictyostelium mucoroides* could not grow in pure-mixed culture with most bacteria upon bouillon or petone agars unless sugar ("glucose, lactose, maltose") were added. Obviously he had made sufficient culture studies to secure a hint as to the relation between culture-medium composition and the growth of *Dictyostelium*. However, he did not record the identity of the bacteria to which he referred; he did not state the concentrations of nutrients used; and he considered together three sugars, each of which imparts distinct properties to a culture medium. Since either of the above factors, when uncontrolled, is capable of altering the cultural picture completely, it is impossible to draw close parallels with the results of the writer's work. Concerning the influence of the reaction of the culture upon the growth and development of the slime mold, he reported that the fruiting structures of *D. mucoroides* in pure-mixed culture with *Bacillus fluorescens liquefaciens* varied in color with the reaction of the culture, being yellow in cultures of alkaline reaction and white in those of acid reaction. But aside from this observation, he apparently did not consider the reaction of the culture of importance in influencing the growth or development of *D. mucoroides*.

Schuckmann (*21*, *22*) cultivated *D. mucoroides* upon a weak beef-infusion agar,[13] a medium not essentially different from a weak peptone agar. His success, the writer believes, was due to the dilute nutrient employed. In this connection, however, it is interesting to note that upon this medium his *Dictyostelium* progressively lost its ability to form fruiting structures (a capacity which could be restored by reculturing it upon horse dung) although the myxamoebae grew well and pseudoplasmodia were regularly formed (*21*). It would seem possible, therefore, that he encountered a culture condition sufficiently toxic to inhibit the formation of sorocarps but not the growth and aggregation of myxamoebae, a condition not unlike that which the writer reports for *D. discoideum* when grown upon media containing moderate amounts of peptone in the absence of sugars which are available to the associated bacteria (e. g., *D. discoideum* in association with *Pseudomonas fluorescens* on S4 medium, table 1).

Brefeld (*3*, *4*), Van Tieghem (*25*, *26*), Olive (*14*), and Harper (*8*, *9*, *10*) did not concern themselves with either the bacterial species that accompanied their cultures or with the reaction of the media they employed. They could neglect these factors and still secure cultures

[13] The medium employed was a so-called amoeba-agar, containing 2 percent agar in 10 parts of beef infusion and 90 parts of distilled water, which had been successfully used by Wasielewski and Kühn (*28*) for cultivating small amoebae belonging to the genus *Valkampfia*.

of the Dictyosteliaceae because they employed media which contained nutrients that were not readily broken down into alkaline or acid products by the bacteria that accompanied their cultures. In an earlier study the writer (*19*) demonstrated that *Dictyostelium discoideum* could grow and develop normally in association with many different bacterial species when cultivated upon hay-infusion agar, a medium poor in accessible nutrients; and in reporting this study the writer stated the following general rule concerning the relationships between the composition of the culture medium and the growth of a member of the Dictyosteliaceae (*19, p. 312*):

> The more dilute a medium is in nutrients, or the less accessible its nutrients are to a large number of different bacterial species, the more probable it is that a member of the Dictyosteliaceae will be able to grow in association with a large number of bacterial species upon that medium.

The correctness of this observation is further confirmed by the results of the present study, for it has been found that *D. discoideum* can grow and develop in an essentially normal manner upon media containing a low concentration of some nutrient, such as peptone, when it grows poorly or not at all in company with the same bacterial associates upon media containing higher concentrations of the same nutrient (table 1).

What, then, is the advantage in employing nutrient-rich media? It is solely for the purpose of securing a more luxuriant growth of the slime mold. And since *Dictyostelium* feeds upon bacteria directly, the primary requisite for a luxuriant growth is a correspondingly abundant food supply. Secondary requisites are, of course, (1) that the colonies of bacteria possess a favorable hydrogen-ion concentration, (2) that the bacterial cells be free from an excess amount of gum, (3) that the cultures be incubated at a favorable temperature, and (4) that a proper degree of humidity be maintained within the culture chambers. Obviously, in order to secure a rich growth of bacteria, the culture medium must contain an abundant supply of nutrients that are readily accessible to the bacteria. At the same time, the more accessible the nutrients are, the more rapidly toxic byproducts will be liberated by the bacteria, and consequently the more carefully the nutrients must be balanced in order that their decomposition products will neutralize each other and not accumulate in quantities detrimental to the slime mold. The use of buffers and pH adjustments can, of course, be substituted for a balance between the carbon and nitrogen sources, but the final aim remains the same, i. e., to prevent the accumulation of decomposition products which in sufficient quantity are toxic to *Dictyostelium*.

SUMMARY

Agar media excellently suited for the laboratory cultivation of *Dictyostelium discoideum* were prepared with peptone and a suitable carbohydrate as nutrients.

A medium containing 1.2 percent each of peptone and a carbohydrate that could be fermented by the associated bacteria provided an especially favorable substratum for the growth of host bacteria and for the subsequent growth and normal development of *Dictyostelium*.

The growth and development of the slime mold upon such media depended upon the composition of the culture medium and the fermentative powers of the associated bacteria.

The growth of the associated bacteria was roughly proportional to the concentration of peptone employed. The incorporation of an available carbohydrate did not enhance the growth of bacteria as a rule, but did insure that the bacterial colonies would be nontoxic to the slime mold. Colonies of host bacteria in the absence of a fermentable carbohydrate became alkaline in reaction because of the accumulation of ammonia liberated from the peptone, and were toxic to *Dictyostelium discoideum*. Colonies of the same bacterial species in the presence of a fermentable carbohydrate in addition to the peptone remained neutral or became mildly acid in reaction because of the neutralization of ammonia by acid products simultaneously produced from the carbohydrate, and were nontoxic to *D. discoideum*.

The growth and development of *Dictyostelium discoideum* were governed by the hydrogen-ion concentration of the bacterial colonies into which the slime mold was introduced. The optimum reaction for the species was pH 6.0 to 6.5, while excellent growth and the formation of normal fruiting structures, or sorocarps, regularly occurred in bacterial colonies of pH 5.0 to 7.0. Outside of this favorable range the growth of the slime mold decreased progressively and its development became consistently more abnormal as the colonies of host bacteria became either increasingly alkaline or more acid in reaction. All growth of the slime mold ceased on the one hand at pH 8.5 to 8.6 and on the other at pH 4.0 to 4.2

The method by which a favorable hydrogen-ion concentration was established in the colonies of host bacteria was apparently unimportant and could be accomplished by the simultaneous and balanced fermentation of both carbon and nitrogen sources by the associated bacteria themselves, or by the addition of free acid to cultures of alkaline reaction, or by the incorporation of sufficient buffers into the media to maintain a favorable reaction regardless of the fermentations by the associated bacteria.

The buffer concentration necessary to maintain a suitable reaction in the colonies of host bacteria depended upon the nutrient composition of the medium, the fermentative capacity of the selected bacterial associate, and the initial pH of the culture medium. Thus peptone media intended for the cultivation of *Dictyostelium discoideum* with *Pseudomonas fluorescens* required either a stronger buffer concentration or a lower initial pH adjustment than media intended for use with *Escherichia coli*, because the former species is the more strongly proteolytic.

In addition to a favorable concentration of hydrogen ions, colonies of host bacteria must have a favorable physical character in order for *Dictyostelium discoideum* to thrive. The myxamoebae could not feed effectively upon bacterial cells encased in gum or slime, although the colonies in which these occurred were wholly nontoxic to the slime mold.

A more nearly optimum cultural environment was required for the organization of myxamoebae into fruiting structures than for their vegetative growth, and in turn a more nearly optimum environment was required for the formation of normal than abnormal sorocarps. *Dictyostelium discoideum* was found to be very sensitive to culture conditions, differences in environment being reflected in the pattern of the sorocarps produced. At the same time, the pattern of sorocarps

served as a useful gage of the favorableness of cultures to this slime mold. Thus *D. discoideum* affords a particularly useful species for investigating the influence of environmental conditions upon the growth and behavior of an amoeboid organism of the soil.

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RELATION BETWEEN CONFORMATION AND ANATOMY OF COWS OF UNKNOWN PRODUCING ABILITY [1]

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PLAN OF THE INVESTIGATION

The subject of body conformation in cattle is of much practical importance, for body form or so-called type to a very large extent provides the basis for selecting individuals for purchase or sale, for mating, for producing purposes, and for judging individuals in the show ring.

Some years ago a plan was evolved to determine such interrelationships as may exist between a cow's conformation, anatomy, and ability to produce milk and butterfat. Conformation in this study refers to the size, shape, and proportions of the outside of the animal body, whereas internal anatomy refers to the size, shape, and proportions of the body cavities and to the size of internal organs as indicated by their weight and measurements.

The problem consists of two main parts. The first is a consideration of the relationship between conformation and internal anatomy. It necessitates measuring the external dimensions of cows in definite units thereby translating conformation into numerical values for purposes of tabulation and analysis, and slaughtering the cows and making a detailed post-mortem study of their internal anatomy. The second part is a study of the relation between both the conformation and the internal anatomy of cows and their producing capacity as previously measured by records of milk and butterfat production.

Arrangements were made with Swift & Co. to conduct in their Chicago packing house a study of the conformation and anatomy of a large number of cows. Since the production records of these animals were not available, the present report deals only with the first part of the problem. Work on the second part is in progress with cows of known producing capacity.

HISTORY OF WORK BEARING ON THE INVESTIGATION

Mierswa (*8*) [3] has reviewed much of the literature pertaining to the relation between body form, anatomy, and performance of farm animals which appeared between 1805 and 1929, and his discussion has been supplemented by that of Becker (*1*) who reviewed the literature published between 1852 and 1911.

Baudement (*8*) found that in oxen of similar age and weight the weight of the lungs decreased as the chest circumference increased.

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[2] The writers are indebted to Swift & Co., Chicago, for providing the animals and the personnel and equipment of their plant which made this study possible; and to the Meat Inspection Division of the Bureau of Animal Industry, U. S. Department of Agriculture, for assistance in obtaining the post-mortem data.

[3] Italic numbers in parentheses refer to Literature Cited, p. 234.

His studies with cattle (*8*) showed also some positive relation between size of lungs and height at withers, larger lungs in young than in older animals of the same live weight, a negative correlation between lung weight and live weight in animals of the same breed, and smaller lungs—absolutely and relatively—in early- than in late-maturing breeds. He concluded that the respiratory activity is smaller in animals showing a larger gain in meat. Considerable importance is attached to his finding that the size of lungs is different in early- than in late-maturing animals and that the animal with excellent fattening qualities is characterized by a big chest circumference, a long cylinder-shaped barrel, low height, deep position of chest bone, short lower legs, small lungs, and little respiratory activity.

Apparently Kögel (*8*) was the first to measure the capacity of the chest cavity and the volume of the collapsed and inflated lungs. He showed that the breastbone was longer in proportion to length of chest and lumbar vertebrae, the chest cavity was larger, the lungs were heavier and larger in proportion to live weight, and the heart was relatively heavier in the late-maturing Merino than in the early-maturing Southdown sheep. Röloff (*8*) arrived at similar conclusions, and pointed out in addition that size of chest depends mainly on length of chest and height of chest cavity. Nathusius (*8*) showed that speed performance in horses increased with decreasing breast circumference and decreasing substance. There is uncertainty, in some cases, as to whether the data representing size or weight of lungs and heart are absolute or relative. If they are based on absolute values, consideration of fat deposition in the region of the chest might temper some of the conclusions. Martiny (*1*) in discussing size of lungs, stressed the importance of their quality and suggested that the small lung may be more efficient than the large one—efficiency being dependent on amount of respiratory surface, degree of elasticity, and development of lung capillaries. He reasoned that lung weight increases with the increase of interstitial framework at the expense of both lung tissue and physiological efficiency. The same view was expressed by Nathusius (*1*) and by Lehmann (*1*). Von Lützow (*7*) stated, as reported by Becker (*1*), that the performance of the lung depends first on the volume of the chest and second on the weight of the lung per cubic centimeter of chest volume—the smaller the weight of the lung the better.

In the case of the heart, however, Colin (*8*) concluded that strength is in direct proportion to heart weight. In two horses of different size he found the lighter lungs in the heavier animal; but the heart was definitely heavier in the heavier horse, except on the basis of live weight, where the difference was negligible. Lehmann (*1*) also contended that increased size in the heart (a muscular organ) is associated with increased function. Von Lützow and Müller (*8*) showed that trotting horses had heavier hearts and lungs than walking horses, in relation to chest width, chest circumference, and live weight. Külbs (*8*) found that exercise definitely increased the relative weight of heart and lungs and the circumference of chest in dogs. The organs, however, showed no microscopic or microchemical changes. Kraemer (*8*) showed an increase in chest circumference in cattle when grazing, although the animals actually lost in live weight. Increase in chest circumference was attributed partly to better growth of the inner

organs as a result of increased activity and partly to the muscular development in the region of the chest as a result of exercise. In discussing Schneider's work with cattle Mierswa (*8*) made the generalization that weight of the inner organs increases with increasing body weight but usually at a slower rate so that the heavier animals do not show a degree of development equal to that of the lighter ones. He indicates also that females usually have proportionately heavier inner organs than males. Seeberger (*15*), as reported by Mierswa (*8*), is credited with showing that in sheep, chest width is negatively correlated with lung size and weight, but that length and depth of chest are positively correlated with size of lungs. He also showed that the lungs were relatively heavier in young than in old animals and relatively heavier in females than in males, and that both heart and lung weights were relatively greater in the late-maturing fertile Franconia sheep than in the early-maturing, easy-fattening Southdowns. From his anatomical studies of swine Semmler (*8*) found that large circumference and width of chest cavity were associated with smaller lungs, but that the greater lengths of chest cavity were associated with greater heart and lung weight. He also showed that brood sows had larger lung volumes than castrated animals used for fattening. Mierswa (*8*) expressed the belief that heavier lungs indicate superior performance, provided the weight is correctly obtained.

Mierswa's comments regarding the report of Swett, Graves, and Miller (*17*) are worthy of note as they confirmed, according to his interpretation, many of the results reported by others. The points he stressed were the greater absolute chest capacity—especially the depth and length—in the lighter dairy cow, whereas the heavier Aberdeen Angus had a heart of approximately the same absolute (smaller relative) weight, and presumably smaller lungs because the chest cavity was smaller.

Becker (*1*) compared the conformation and anatomy of two breeds of cattle, paying particular attention to the external and internal measurements of the chest and the size of heart and lungs. The Simmentals and Oldenburgs studied were of about the same live weight (average 606 and 596 kg respectively) and measured almost the same in height and in circumference and width of chest. The Oldenburgs, however, were much longer and considerably deeper in the chest and had greater depth of thoracic cavity, but the breeds differed little in width of thoracic cavity. The thoracic index (external depth of chest÷external width of chest), and the heart weight and lung weight—especially the latter—were greater in the Oldenburgs. Moreover, the heart and lung indexes I, II, and III (weight of organ÷circumference, depth, and width, respectively, of thoracic cavity) averaged greater in every case for the Oldenburgs, although the difference was less marked for the heart than for the lungs. The differences varied somewhat at different ages. In relation to both live weight and carcass weight the heart and lungs were larger in the Oldenburgs. In the Simmental breed the higher values were found at early ages, whereas in the Oldenburg breed the values increased with age. The greatest differences therefore occurred with the more advanced ages. These results indicate that the large heart and lungs are found in animals having chests that are deep in proportion to their width.

Mierswa's own studies (*8*) have a very close bearing on the relation of external form to internal anatomy. In comparing milk-, intermediate-, and meat-type breeds of cattle Mierswa found definite associations between external body measurements, internal chest measurements, and the size of the heart and lungs. This was noted particularly in chest lengths and chest depths, while chest width was found to be of little if any significance. In animals with equal chest index (average of the three chest dimensions) and with equal live weight, the thoracic capacity was greater in females than in males. Size of chest cavity was greatest for the dairy-type, less for the intermediate-type, and least for the beef-type breeds. In proportion to live weight the heart was heavier in females than in males, and among the females was heaviest in the milk-type and lightest in the meat-type breeds. No general relations were found in studying the relative lung weight. Mierswa pointed out that all substantial differences shown in the relation between outer and inner chest measurements and between external measurements and heart and lung development, are dependent on the ability of the different breeds and types to put on flesh or fat around the chest. Mierswa's findings were in some respects contrary to those of Korreng (*5*), which indicated that relative heart weight was greater in the male than in the female and lung weight was greater in the female.

Von Lützow (*7*) found that volume of thoracic cavity and lung weight were greater in slow horses than in fast ones, even though the heart weight was proportionately larger in fast horses. Müller (*11*, *12*) disagreed with some of Von Lützow's conclusions, pointed out inaccuracies resulting from estimating volume of thoracic cavity, and stated that lung weight cannot be estimated from its volume on account of variation in its specific gravity. Seeberger (*15*) concluded from his study of sheep that a thoracic cavity with a large index (high width in relation to depth) is correlated with a smaller lung.

A comprehensive study of the effect of age and plane of nutrition on body form, internal anatomy, and chemical composition of the different parts of the body, begun at the Missouri Agricultural Experiment Station in 1906 (*9*), shows the anatomic structure of the animals when slaughtered. The ratio of empty weight to live weight was greatest at birth, least at 8½ months of age, and intermediate at 4 years. Animals on low planes of nutrition had low ratios. The ratio of carcass weight to live weight decreased with age to 8½ months and then increased to a maximum at 3 to 4 years of age. The better-fed animals had the highest ratio. On the basis of empty weight the carcass weight increased continuously with age up to 3 or 4 years. Hide and hair, heart, brain and spinal cord, and intestine length decreased in relation to empty weight with an increase in age and fatness. The weights of blood, lungs, stomachs, intestines, liver, kidneys, spleen, and pancreas, however, increased in relation to empty weight up to about 8½ months, after which the relation showed a decrease. The stomachs and liver showed marked retardation on the low planes of nutrition. The limited number of animals and the fact that they represented different degrees of fatness and were slaughtered at different ages, made any attempt to correlate organ size with body dimensions impracticable. A comparison of the anatomy of cows representing different degrees of fatness, on the basis of percentage of empty

body weight (*10*) showed that the fat cows had low values for weights of carcass, heart, lungs, brain and spinal cord, stomachs, intestines, and spleen; high values for offal fat and liver; and approximately the same values for hide, blood, and pancreas. With fattening, most of the internal organs decreased or remained constant in relation to empty body weight.

The Bureau of Dairy Industry (*17*) has shown that, although a highly specialized dairy cow differed greatly from a highly specialized beef cow in external form, the internal organs did not differ sufficiently in size and weight to indicate significant differences in function. The skeletal structure of the cows was generally similar, indicating the great effect of fleshing on external body form. The comparison of a cow and a bull of the same breed (*18*) also showed that the external form of the two animals differed greatly in certain particulars, especially in width and circumference of body and general heaviness of the anterior parts, but that there was little difference in body depth or scale. Only two of the internal organs—the thyroid and the spleen—were larger in relation to empty body weight in the bull than in the cow. The skeletons of the cow and bull were similar except for the head, the cervical vertebrae, and the shape of the pelvis. The differences in body form in this case also were due chiefly to differences in muscular development and fleshing. A study of the comparative conformation, anatomy, and udder characteristics of cows of certain beef and dairy breeds (*19*) showed marked differences in body form. Cows of the Hereford, Holstein-Friesian, and Aberdeen Angus breeds were found to be generally similar in skeletal structure. The most marked differences in external body proportions were associated with measurements most directly affected by fat deposition. There was a tendency for the internal organs to be largest in the Holsteins. In relation to empty body weight, however, in most cases the Herefords had the largest, and the Aberdeen Angus the smallest internal organs. Apparently as fleshing increases the total size or weight of the animal structure, the organs become smaller in relation to empty body weight.

A recent publication [4] having a particularly close bearing on the present work is a progress report on the study of conformation and anatomy of dairy cows in relation to their producing capacity. This work is being conducted by the Bureau of Dairy Industry in cooperation with a number of State agricultural experiment stations. As the data are not yet sufficient in number, correlations were not given and conclusions were not drawn. The report gave the average, maximum, and minimum weights and measurements, together with averages for large, medium, and small cows in five different breed groups. Data were given for about 120 different ante-mortem and post-mortem items, and 593 cows having records of production were included.

Joseph (*4*) found that the ratio showing the number of grams of heart weight per kilogram of live weight varies from 2.36 and 2.76 for the tame and wild rabbit, respectively, to 11.55 for the deer and 25.64 for the thrush. Other ratios are 4.52 for the pig, 3.83 for the cow, 5.85 for the ewe, 5.81 for the horse, and from 7.43 to 9.71 for dogs. There seems to be some relation between physical activity and ratio of heart weight to live weight.

[4] SWETT, W. W., MATTHEWS, C. A., MILLER, F. W., and GRAVES, R. R. VARIATIONS RECORDED IN THE STUDY OF THE CONFORMATION AND ANATOMY OF 593 DAIRY COWS HAVING RECORDS OF PRODUCTION. U. S. Bur. Dairy Indus., BDIM—589, 23 pp. 1937. [Mimeographed.]

Stewart (*16*) studied the relation of organ size to body weight and body-surface area in dogs. He found that the spleen and heart weights were in general proportional to body weight, whereas the liver, stomach, intestine, kidney, and lung weights were more nearly proportional to body-surface area. Roaf (*14*), on the contrary, found that the ratio of heart and liver weights to body weight in cats varied independently of body weight, whereas the ratio of kidney weight to body weight varied directly with body weight.

Kraemer (*6*) studied the skull capacity and brain weight of domestic and wild animals, fishes, and human beings. He found the brain to be generally larger in males than in females and he found also greater absolute brain weights in large than in small animals. As a rule the smaller individuals had the larger brains in relation to their live weight.

EXPERIMENTAL PROCEDURE

METHODS OF OBTAINING ANTE-MORTEM DATA

The usual procedure in the stockyards at the time these data were obtained was to unload the cattle in the morning and to feed and water them immediately. Buyers for the packing company then proceeded to make purchases for the day. From such purchases cows of certain types were selected for this study, tagged for identification, and weighed individually during the late morning or early afternoon of the same day. The cows selected represented a wide range in type or conformation, an effort being made to include not only the deep, wide, well-developed individuals, but also the narrow, shallow animals, which theoretically should be deficient from the standpoint of constitution and capacity. Most of the cows selected showed definite characteristics of some dairy breed.

Usually the cows were held in small stockyard pens during the rest of the day and night, with access to water but not to feed. In most instances they were driven to the larger packing-house pens early the following morning, although a few were moved in the late afternoon of the day of purchase.

During the morning following the day of purchase, the external body measurements (ante-mortem data), consisting of 17 body measurements and a number of observations, were obtained for each animal. The slaughtering of these animals took place on the afternoon of the same day except in a few instances when they were slaughtered just before noon, immediately after the completion of the ante-mortem measuring. Approximately 24 hours elapsed, therefore, between last feeding and the time of measuring; approximately 18 hours between weighing and measuring; and about 24 hours between weighing and slaughtering.

Considerable individual variation was found in the readiness with which the cows permitted handling. Some evidently had come from dairy herds and were accustomed to daily handling. Others apparently had not been handled regularly, and some difficulty was experienced in holding them in the desired position for measuring. In such cases incorrect position of the feet may have slightly affected such measurements as height at withers and some of the dimensions of the chest. However, all the ante-mortem data except those for live weight were obtained by one person and are as uniform and accurate as could reasonably be expected.

Figure 1 illustrates the landmarks, or points of anatomy, at which the different external body measurements were taken.

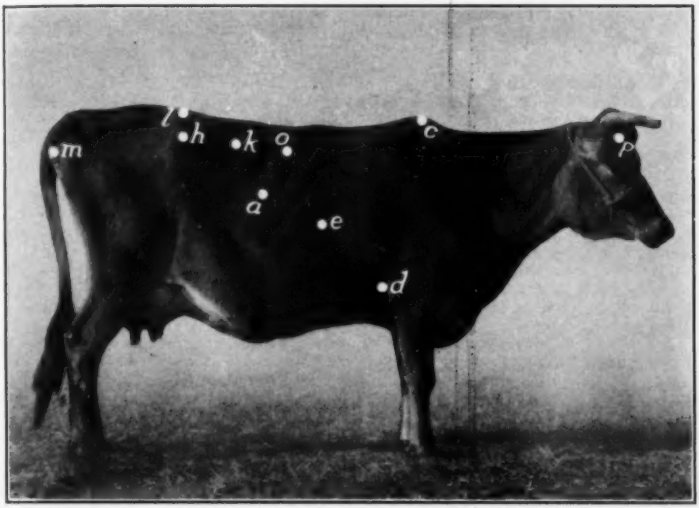

FIGURE 1.—Landmarks, or points of anatomy, used in making the body measurements. See text for explanation of points.

A list of the ante-mortem items recorded, together with details concerning the methods employed in obtaining them, are given below. Body weight is recorded in pounds and body measurements in centimeters. The first few items, which are descriptive rather than measurements of conformation, do not enter into the tabulations.

ANTE-MORTEM ITEMS RECORDED

Identification of animal.
General source (State from which animal was received).
Breed.
General type.
General condition.
Handling quality of hide at center of thirteenth rib. Taken at *a* (fig. 1).
Thickness of fold of hide at center of thirteenth rib. Measured with vernier caliper at *a*.
Live weight.
Height at withers. The point represented by *c* is directly above and directly in line with the front leg when squarely placed.
Depth of fore chest. Taken with vertical caliper through the vertical transverse plane passing just to the rear of the point of the elbow (ulna), at *d*.
Depth of rear chest. Taken with vertical caliper through the vertical transverse plane passing through the center of the tenth rib (the fourth rib from the rear), at *e*.
Depth of paunch. Taken with vertical caliper through the vertical transverse plane passing through the center of the thirteenth (or last) rib at *a*.
Width of fore chest. Taken with horizontalcaliper through the vertical transverse plane passing through *d*.
Width of rear chest. Taken with horizontal caliper through the vertical transverse plane passing through *e*.

Width of paunch. Taken with horizontal caliper through the vertical transverse plane passing through *a*.

Width of hips. Taken with horizontal caliper touching lateral points of hips (tuber coxae) at *h*.

Width of loin. Taken with horizontal caliper touching lateral edges of center of loin at *k*.

Length of loin. Taken with tapeline from the lateral face of hip point (tuber coxae) forward to the highest point along the posterior edge of the thirteenth rib to which a finger or thumb can be forced (*h* to *o*).

Length, withers to hips. Taken with a tapeline from withers to a median point on the spine midway between tops of hip points (*c* to *l*).

Length, hips to pinbones. Taken with tapeline from a point on spine midway between tops of hip points to posterior tip of pinbone (from *l* to a point n ar to and directly above *m*).

Length, withers to pinbones. Sum of lengths from withers to hips and from hips to pinbones.

Circumference of fore chest. Taken with tapeline through the vertical transverse plane passing through *d*.

Circumference of rear chest. Taken with tapeline through the vertical transverse plane passing through *e*.

Circumference of paunch. Taken with tapeline through the vertical transverse plane passing through *a*.

Width of head. Taken with tapeline between extreme lateral edges of forehead at narrowest point (*p*), midway between eyes and the poll. (In measuring width of head, since the edges of the skull are somewhat posterior to the frontal face of the forehead, the tape curves over the face and registers a value slightly in excess of the true width at *p*.)

METHODS OF OBTAINING POST-MORTEM DATA

The cows selected for the day's test were slaughtered at the end of the day's killing, after the slaughterhouse routine had been stopped for a brief period. The rapidity of killing was somewhat reduced for the test group, but otherwise the usual procedure was followed, except for slight variations necessitated by the weighing and measuring of the organs and body parts. Before the organs and parts of an animal's body were separated for distribution to the different divisions of the slaughterhouse, each was given an identification mark corresponding to one on a tag affixed to the ear. Nearly all organs of the body were weighed, and some were measured, as they passed through the different divisions of the packing house.

The work of collecting data required the services of from 15 to 20 men of the Bureau of Animal Industry, in addition to the regular employees of the packing house, for a part of each day over a period of nearly a month. Selected employees were stationed in groups at each of the places where data were to be obtained. Each group was equipped with special scales and other equipment used in obtaining the data. One member of the group was assigned to record the data on each of six or more groups of organs, as the other members prepared them. In this manner the data were collected almost simultaneously and in a brief period of time. In most cases identification of the different organs and parts of each animal was accomplished readily as they reached the different processing points in the slaughterhouse. However, some items for some animals had to be eliminated because of pathological conditions or because of failure to identify the organ or part with any particular individual. Thirty-one post-mortem items were recorded in addition to several internal measurements of the thoracic cavity. Complete post-mortem data were obtained on more than 200 cows and partial data on more than 350 cows.

The nature of the organ or part and the relative difficulty involved in its preparation may have affected the results slightly. Some organs were more easily and uniformly weighed or measured than others. For example, in some cases a small quantity of blood was lost; the pancreas, an irregularly shaped organ, was subject to some variation in preparation; and there was some lack of uniformity in separating the brain from the spinal cord. The weight of the pituitary body is omitted from all tabulations because the scale used did not permit weighing in sufficiently fine units, and because in its preparation an excessive quantity of membrane was left attached in some cases. In the usual procedure of handling the stomachs in the packing house, the omasum was "hashed" with its contents and made into tankage. In order to determine the weight of the omasum, 50 omasa were hashed but recovered and washed for weighing, the average weight for the 50 being added to the total for the other three divisions of the stomach for each cow to make up the total empty weight of stomach. A slight error is introduced in obtaining the total stomach weight in this manner. Moreover, the necessity for using six or more groups of individuals, in taking and recording the post-mortem data, and the fact that the personnel of some of these groups changed somewhat from day to day, may have introduced slight variations in methods, but the data are believed to be satisfactorily uniform and accurate.

The following is a descriptive list of the items for which post-mortem data were recorded, although not all of them are included in the tabulations that appear later in this paper.

POST-MORTEM ITEMS RECORDED

Identification of animal.
Live weight (pounds).
Weight of blood (pounds).
Weight of hide (pounds).
Weight of caul fat (pounds).
Weight of fetus, if cow was pregnant (pounds).
Weight of lungs (after separation from trachea) (pounds). Taken for (1) right lung, (2) left lung, and (3) total, both lungs.
Weight of heart after removal of fat (pounds).
Circumference of heart after removal of fat (centimeters). Taken (1) in a plane parallel to and close to base, and (2) over the apex in a plane passing through the apex.
Weight of thoracic fat (pounds).
Weight of liver after removal of gall bladder and fat (pounds).
Weight of intestines and contents. Taken as removed, with fat and mesentery attached (pounds).
Weight of intestine fat and mesentery (pounds).
Weight of empty intestines (pounds). Taken for (1) small intestine, (2) large intestine, and (3) total of both.
Weight of contents of intestines (pounds). Represents difference between weight of intestines with contents and weight of empty intestines and fat.
Length of intestines (feet). Taken for (1) small intestine, (2) large intestine, and (3) total of both.
Weight of stomach and contents with fat removed (pounds).
Weight of stomach fat (pounds).
Weight of stomach after being emptied and washed (pounds). Taken for (1) rumen, reticulum, and omasum;[5] (2) abomasum, and (3) total for all four divisions.
Weight of contents of stomach (pounds) (calculated by difference).
Weight of total abdominal fat (pounds).

[5] Weight of omasum not determined for each cow. Average of 50, cleaned and weighed together, used in all cases.

Weight of pancreas [6] (ounces).
Weight of spleen (pounds).
Weight of brain (ounces).
Weight of pituitary body [7] (ounces).
Measurements of thoracic cavity of dressed carcass, as follows:

(1) Maximum dorsoventral diameter of thoracic cavity (centimeters). Taken from spinal cord at each thoracic vertebra to sternum or to diaphragm.
(2) Maximum length of thoracic cavity (centimeters). Taken with tape-line from the extreme posterior point, at attachment of diaphragm, to the anterior edge of the first rib.
(3) Maximum internal lateral width of thoracic cavity (centimeters). A measure of the concavity of the split thorax, taken at the seventh rib.

Weight of carcass (pounds).

SUPPLEMENTAL VALUES CALCULATED FROM ANTE-MORTEM AND POST-MORTEM DATA

A number of supplemental values have been calculated from the original ante-mortem and post-mortem data to facilitate the study of comparative conformation and anatomy. Many of the supplemental values represent body proportions in the form of ratios or percentages. Those which represent body-surface area; volume of barrel; cross-section areas of fore chest, rear chest, and paunch; and volume of thoracic cavity are considered to be close approximations and reasonably significant, but no claim is made as to their exactness. Following is a discussion of the derivation of the supplemental data.

(1) The body-surface area is supposed to be closely related to metabolism. For this reason it was calculated for all animals by means of the formula of Hogan and Skouby (*3*): $S=W^{0.4}\times L^{0.6}\times K$, in which S=surface area in square centimeters, W=live weight in kilograms, L=length of body in centimeters, and K=217, a constant value for cattle. The live weight as taken in pounds has been converted to kilograms. The length from the withers to a line between the hips added to the length from a line between the hips to the pinbones, gives the total length of the body (withers to pinbones).

(2) Legginess is a term frequently used to denote the proportion of the animal's height located between the ventral surface of the torso and the ground, or the proportion of the length of the legs to the total standing height of the animal. In this study legginess is determined by subtracting the depth of fore chest from the height at withers and dividing the difference by the height at withers.

(3) Cross-section area of fore chest, a term applied to the area of an ellipse having as its axes the two maximum diameters of the fore chest, is used to represent the cross-section area of the body through the fore chest. This value is derived by multiplying the depth of fore chest by the width of fore chest, and the product by 0.7854. This area is undoubtedly somewhat in excess of the actual cross-section area of the fore chest, because depressions occur in many cows, posterior to the scapula and ulna.

[6] Dissection not perfectly performed. Accuracy of weights subject to some question.
[7] Scales used were not sufficiently delicate for weighing so small an organ. Completeness of dissection subject to question.

(4) The cross-section area of rear chest is the product of the depth of rear chest, the width of rear chest, and the factor 0.7854. This appears to give a close approximation of the cross-section area through this plane as the contour of the body is usually reasonably regular at that point.

(5) The cross-section area of paunch is the product of the depth of paunch, the width of paunch, and the factor 0.7854. A close approximation of the cross-section area at that point also should be obtainable in this manner because of the fairly regular contour lines at that plane in most cows.

(6) The volume of barrel, which is generally associated with capacity for feed consumption and producing ability in the cow, is calculated by multiplying the average of the cross-section areas of the fore chest and paunch by the linear distance from the withers to a median point on the spine midway between the tops of the hip points.

(7) Thoracic index, a term used in referring to the "build" of humans, is the ratio of the depth to width of chest and is determined for cattle by dividing the depth of fore chest by the width of fore chest.

(8) The rear-chest index is the ratio of the depth of rear chest to width of rear chest.

(9) The abdominal index, which is the ratio of depth to width of paunch, is determined by dividing depth of paunch by width of paunch.

Obviously a high index for fore chest (7), rear chest (8), or paunch (9) indicates that the animal was deep in relation to width of body at the point measured. The indexes may be used, therefore, as a basis for classifying animals, with regard to the proportions of the trunk.

(10) Wedge shape, which refers to the angular form of the body, is generally considered desirable in dairy cows. For comparative purposes it can be expressed in terms of the relation of the fore chest to the paunch dimension in depth, width, or circumference. This relation, for each of these dimensions, is shown as a simple ratio of paunch measurement to fore-chest measurement obtained by dividing the former by the latter. It is also shown in actual units of measurement as the difference between the chest measurements and the corresponding paunch measurements.

(11) Volume of thoracic cavity is difficult to calculate because of its irregular shape and outline and the flexibility of the diaphragm, and at best it can only be approximated. At the same time some measure of the thoracic capacity is desirable as it represents the space available for the heart and lungs. An attempt has been made to calculate the volume or capacity of the thoracic cavity on the basis of two frustums of cones having a common base. It is apparent that these figures cannot be truly conical in shape because their bases are elliptical rather than circular. The formula for determining the area of an ellipse was used in calculating the base areas, and the formula for calculating the volume of a frustum of a cone was then applied.

The size of the forward or anterior frustum is found as follows: Complete measurement of the skeleton of a dairy cow shows that the axes of the thoracic cavity at the extreme anterior point (first rib) are approximately in the proportion of 2 to 1. The area of the anterior

base A of the anterior frustum is approximated when the depth of cavity at the first thoracic vertebra is multiplied by one-half itself, and this product is multiplied by 0.7854. The posterior base C of the anterior frustum, which is the common base of both frustums, has as axes the maximum interior depth and the interior lateral width of the thoracic cavity at or near the seventh rib. The maximum interior depth does not necessarily come at exactly the seventh-rib plane, but is not far distant. The area of the common base C therefore is the product of these two axes times 0.7854. The altitude of the anterior frustum is assumed to be one-half the maximum length of the thoracic cavity, as measured from the anterior edge of the first rib to the posterior point of attachment of the diaphragm. The volume of the anterior frustum is:

$$(\text{Base } A + \text{base } C) + \sqrt{\text{base } A \times \text{base } C} \times \frac{\text{length of thoracic cavity}}{6}.$$

The volume of the posterior frustum is determined similarly. In this frustum base C, which is common to both frustums, represents its anterior base. Its posterior base, P, which is indefinite in shape and outline, is assumed to have axes in the proportion of 2 to 1, with the lateral axis twice the vertical one. The vertical axis is measured as the dorsoventral diameter of the cavity at the most posterior vertebra in the cavity. To obtain the area of base P this last dorsoventral, or vertical, dimension is multiplied by twice its value, and the product by 0.7854. The volume of the posterior frustum is:

$$(\text{Base } C + \text{base } P) + \sqrt{\text{base } C \times \text{base } P} \times \frac{\text{length of thoracic cavity}}{6}.$$

The volume of the entire thoracic cavity is the sum of the volume of the anterior and posterior frustums.

(12) Percentage of heart weight to live weight is determined by dividing the weight of heart by the live weight.

(13) Percentage of lung weight to live weight is determined by dividing the total weight of lungs by the live weight.

PRESENTATION OF DATA

VARIATION

The variation in the magnitude of the range in the different measurements was marked, but the variation in the post-mortem data appeared to be much greater than in the ante-mortem data. The results of an analysis of the degree of variation in the ante-mortem and in the post-mortem data are given in tables 1 and 2.

Tables 1 and 2 enumerate the items studied and show the units in which the measurements were recorded. Averages of the lowest five and the highest five values instead of the lowest and highest single values were used to avoid attaching undue importance to individual extremes in showing the range of the data obtained. One column gives the arithmetic average of the entire lot of measurements for each item. Another column shows the median or midmost measurement in the entire population. If there were 361 measurements for

a given item, the median would be the one hundred and eighty-first value from both the highest and the lowest. In the case of 362 animals in the group, the median would be halfway between the one hundred and eighty-first and the one hundred and eighty-second values, counted from either the highest or the lowest. The standard deviation with its probable error is given to show the actual dispersion of the individual measurements for each item, and the coefficient of variation with its probable error is given to show the comparative measure of dispersion for different items.

TABLE 1.—*Variations in the ante-mortem data obtained from cows slaughtered in a packing house*

Item [1]	Minimum measurements (average of 5 lowest)	Maximum measurements (average of 5 highest)	Arithmetic average (of all measurements)	Median (the midmost measurement)	Standard deviation and probable error	Coefficient of variation and probable error
Data as obtained:						
Thickness of hide (361) [1] centimeters	0.51	1.15	0.78	0.77	0.12±0.00	15.86±0.40
Live weight (362) pounds	738	1,424	1,059.67	1,070.00	132.59±3.32	12.51± .31
Height at withers (362) centimeters	117.30	141.20	129.02	129.00	4.82± .12	3.73± .09
Depth of fore chest (362) do	65.50	81.40	72.61	72.50	2.99± .07	4.11± .10
Depth of rear chest (362) do	64.40	80.80	72.35	71.50	3.30± .08	4.56± .11
Depth of paunch (362) do	62.30	82.90	72.83	73.00	4.01± .10	5.50± .14
Width of fore chest (361) do	33.70	50.90	41.47	41.50	3.35± .08	8.08± .20
Width of rear chest (362) do	45.60	65.00	55.93	56.00	3.91± .10	7.00± .18
Width of paunch (362) do	45.50	70.00	58.02	58.00	4.82± .12	8.31± .21
Width of hips (362) do	45.50	61.90	53.81	54.00	2.96± .07	5.50± .14
Width of loin (361) do	29.20	41.70	35.78	36.00	2.33± .06	6.52± .16
Length of loin (334) do	26.00	45.00	34.29	34.00	3.25± .08	9.47± .25
Length, withers to hips (362) do	81.20	105.20	93.51	94.00	4.54± .11	4.85± .12
Length, hips to pinbones (361) do	45.20	58.20	50.80	51.00	2.53± .06	4.97± .12
Length, withers to pinbones (361) centimeters	129.80	160.60	144.31	144.00	5.98± .15	4.15± .10
Circumference of fore chest (362) centimeters	165.80	210.20	186.41	186.00	8.37± .21	4.49± .11
Circumference of rear chest (361) centimeters	180.60	233.20	206.90	207.00	10.72± .27	5.18± .13
Circumference of paunch (362) do	177.80	242.20	211.56	211.00	12.38± .31	5.85± .15
Width of head (362) do	19.40	26.60	22.91	23.00	1.56± .04	6.82± .17
Calculated values showing body bulk:						
Body-surface area (361) square centimeters	41,666	59,909	50,615	50,674	3,454±87.00	6.82± .17
Volume of barrel (361) cubic centimeters	185,853	357,599	266,990	267,546	34,714± 871	13.00± .33
Cross-section area of fore chest (361) square centimeters	1,823.62	3,124.91	2,368.24	2,353.84	247.62± 6.22	10.46± .26
Cross-section area of rear chest (362) square centimeters	2,395.63	3,990.38	3,182.50	3,192.65	330.40± 8.28	10.38± .26
Cross-section area of paunch (362) square centimeters	2,298.71	4,300.46	3,327.22	3,326.17	404.13±10.13	12.15± .30
Calculated values showing body proportions:						
Legginess (362)	0.378	0.485	0.437	0.438	0.02± 0.00	4.08±0.10
Thoracic index (361)	1.49	2.07	1.76	1.74	.13± .00	7.42± .19
Rear-chest index (362)	1.135	1.508	1.298	1.295	.07± .00	5.73± .14
Abdominal index (362)	1.067	1.499	1.261	1.259	.09± .00	6.98± .17
Wedge shape: [2]						
Depth, ratio (362)	.878	1.137	1.004	1.007	.04± .00	4.29± .11
Width, ratio (361)	1.170	1.661	1.403	1.402	.10± .00	7.21± .18
Circumference, ratio (362)	1.032	1.254	1.134	1.134	.04± .00	3.93± .10
Depth, units (362)	−9.20	+8.20	+.22	+.50	3.03± .08	13.65± .34
Width, units (361)	7.50	26.60	16.59	16.50	3.80± .10	22.90± .57
Circumference, units (362)	5.80	46.80	25.08	25.00	8.26± .21	32.93± .83

[1] Numbers in parentheses show number of animals represented.
[2] For explanation of items see p. 209.

TABLE 2.—*Variations in the post-mortem data obtained from cows slaughtered in a packing house*

Item [1]	Minimum measurements (average of 5 lowest)	Maximum measurements (average of 5 highest)	Arithmetic average (of all measurements)	Median (the midmost measurement)	Standard deviation and probable error	Coefficient of variation and probable error
Weight of blood (362)—pounds	25.05	65.40	40.69	40.00	7.33±0.18	18.02±0.45
Weight of hide (353)—do	47.80	86.60	64.00	63.00	7.60± .19	11.87± .30
Weight of lungs (234)—do	4.66	11.57	7.36	7.18	1.28± .04	17.40± .54
Weight of heart (260)—do	3.09	5.80	4.42	4.44	.59± .02	13.27± .39
Circumference of heart at base (237)—centimeters	34.40	53.68	44.12	44.00	3.58± .11	8.11± .25
Circumference of heart over apex (237)—centimeters	41.40	61.00	50.42	50.00	3.49± .11	6.93± .21
Weight of liver (244)—pounds	8.52	18.20	12.91	13.00	1.85± .06	14.30± .44
Weight of empty intestines (277)—do	16.22	29.71	21.36	21.12	2.60± .07	12.15± .35
Length of intestines (297)—feet	106.00	180.50	139.46	137.00	13.45± .37	9.64± .27
Weight of empty stomach (311)—pounds	26.90	52.60	38.84	38.60	4.81± .13	12.40± .34
Weight of pancreas (268)—ounces	1.48	16.75	7.46	7.06	3.26± .10	43.76±1.27
Weight of spleen (245)—pounds	1.16	2.69	1.77	1.75	.31± .01	17.54± .53
Weight of brain (321)—ounces	10.40	19.18	15.60	15.62	1.51± .04	9.66± .26
Weight of thoracic fat (230)—pounds	1.53	7.20	3.25	2.97	1.18± .37	36.39±1.14
Weight of abdominal fat (316)—do	8.90	64.90	25.49	23.00	10.95± .29	42.97±1.15
Depth (dorsoventral) of thoracic cavity (274)—centimeters	43.80	56.20	49.59	49.00	2.23± .06	4.50± .13
Length (anterior-posterior) of thoracic cavity (274)—centimeters	57.00	80.20	70.69	71.00	4.12± .12	5.83± .17
Width (lateral) of thoracic cavity (270)—centimeters	30.30	41.60	35.59	36.00	2.35± .07	6.60± .19
Weight of carcass (358)—pounds	340.20	789.00	537.64	537.00	84.87±2.14	15.78± .40
Volume of thoracic cavity (267)—cubic centimeters	53,574	102,860	75,087	74,749	10,092.29±294.60	13.44± .39
Heart weight per 100 pounds of live weight (260)—pounds	0.28	0.55	0.41	0.41	.05±0.00	12.15± .36
Lung weight per 100 pounds of live weight (234)—pounds	.45	1.06	.69	.67	.12± .00	17.26± .54

[1] Numbers in parentheses show number of animals represented.

VARIATION IN ANTE-MORTEM DATA

Nineteen of the items in table 1 represent the data in units of weight or measurement as obtained on the living animal. Of these 19 items 17 are primarily measurements of skeletal size, 1 (live weight) is a measure of mass, and 1 (thickness of hide) presumably has no relation to either skeletal size or mass. In addition, there are 5 calculated items designed to show a measure of body bulk and 10 calculated items which show body proportions.

The arithmetic average and the median are almost identical for each item in the ante-mortem data, indicating a normal distribution which centers near the average.

The standard deviation, of course, tends to increase in magnitude with an increase in the magnitude of the measurement it represents. The significance of the standard deviation for each item in relation to the arithmetic average for that item, is shown by the coefficients of variation which are given in table 1, and discussed in the following paragraphs.

In the group of 19 items of weights and measurements, thickness of hide and live weight have the highest coefficients of variation. Length of loin, with a coefficient of 9.47, has the next highest, followed by the five measurements for width of body. The items in this group showing the smallest coefficients of variation are the height at withers,

3.73, depth of fore chest, 4.11, and length from withers to pinbones, 4.15. In general, the items in this group that indicated depths, with an average of 4.72 for the coefficients, lengths, with an average of 5.86, and circumferences, with an average of 5.17, were less variable than the items that indicated width of body, for which the coefficients averaged 7.04, with the single exception of the length of loin.

The coefficients of variation for the calculations showing body bulk or size, and those for the calculations showing body proportions, were greater on an average than those for items indicating skeletal size. The average of the coefficients for each group was 10.56 for the calculations showing body bulk, 10.91 for the calculations showing body proportions, and 5.83 for the measured items showing skeletal size. The greatest variations were found in connection with the measurements affected by condition of flesh and "fill" of the animal; and the least in connection with body height and the body lengths that represent body scale.

Variation in Post-Mortem Data

Variations have been studied for 22 post-mortem items. These include 19 items based on weights or measurements as taken, 2 ratios that show the size of heart and lungs in relation to live weight, and 1 calculated value giving an approximate measure of the volume of the thoracic cavity. Although the two lobes of the lungs, the large and small intestines, and the different divisions of the stomachs were weighed or measured separately, only the totals for each of these organs are given in table 2.

As in the case of the ante-mortem data, the arithmetic average and the median are almost equal for post-mortem items, indicating a normal distribution centering on the mean.

The three highest coefficients of variation are those for weights of pancreas, abdominal fat, and thoracic fat, and the three lowest are those for the depth, length, and width measurements of the thoracic cavity. Just why the variation in the weight of pancreas was so great cannot be explained. It was probably due in part to differences in trimming, but data secured in another project indicate that there is a great variation in the weight of this organ. The next group in the order of magnitude of their coefficients of variation includes the weights of blood, spleen, lungs, lungs per 100 pounds of live weight, and carcass, with coefficients ranging from 18.02 to 15.78. This group is followed by the weight of liver, volume of thoracic cavity, weights of heart, empty stomachs, empty intestines, heart per 100 pounds live weight, and hide, with coefficients ranging from 14.3 to 11.87. The variations are greater in the weights of the empty intestines and the heart than in the measurements of those organs. The coefficients of variation for the heart and lungs are also much larger than those for the width, length, and depth of thoracic cavity. The coefficients for these last three measurements are quite similar to those for the ante-mortem measurements for depth, width, and circumference of chest. The coefficient of variation for the weight of brain is greater than that for width of head in the ante-mortem data, and the same is true of the coefficients for other measurements of organs as compared with those for skeletal size. For all of the 19

post-mortem items of weight and measurement, the average of the coefficients of variation was 16.16, as compared with an average of 5.83 for the 17 ante-mortem measurements of skeletal size.

In a previous publication (*19*) the post-mortem data were divided into seven groups on the basis of the functions of the organs and body parts, to determine the extent to which those functions are associated with anatomical differences. The items that were included in this study were grouped according to the same plan, as follows: (1) Body-size group, which includes live weight,[8] weights of hide and dressed carcass, and measurements of carcass; (2) nervous-system group, which includes weight of brain; (3) circulation and respiration group, which includes weights of blood, lungs, and heart, and measurements of the heart; (4) digestion group, which includes weights of liver, pancreas, intestines, and stomach, and intestine measurements; (5) urogenital group (not included); (6) endocrine-gland group, which includes weight of spleen; and (7) visceral-fats group, which includes weights of abdominal and thoracic fats. The highest variations occurred in the visceral-fats group, the second highest in the digestion group, and the lowest in the nervous-system (brain) and body-size groups.

RELATION BETWEEN EXTERNAL CONFORMATION AND INTERNAL ANATOMY, AND INTERRELATIONS BETWEEN ANTE-MORTEM AND BETWEEN POST-MORTEM ITEMS

Owing to the large number of variable items under consideration (34 ante mortem and 22 post mortem), the determination of interrelations for all possible combinations of the separate items would be virtually an endless task. However, it appeared practicable to study the relation of the weight or measurement of certain vitally important organs representing different functions and anatomical locations, to the external body measurements (ante-mortem data) and to the size of the other internal organs and body parts (post-mortem data); to determine what relation the live weight of the animal bears to the different measurements of conformation and to the weight and measurement of the internal organs and body parts; and to study the interrelation between some of the external body measurements and between some of the different items of internal anatomy.

In addition to live weight, the weight of heart, the weight of lungs, and the length of intestines were chosen for correlation studies. The heart and lungs were chosen because they have long been associated with theories concerning the vitality and performing ability of animals, and also because their weights can be determined readily and definitely. The length of intestines was chosen because it can be measured with a high degree of accuracy and it seemed desirable to include in the study a representative part of the digestive system. It was important to include live weight as one of the items of study in order to determine the extent to which the size of the different body parts may be related to, or independent of, the size of the animal.

More than 40 correlation coefficients were determined for each of these four items (heart weight, lung weight, intestine length, and live weight). The results are given in four tabulations, and the correlation coefficients in each tabulation are arranged in the order of descending magnitude. A coefficient of 0.500 or more is generally considered

[8] Since only one live weight is available for each cow used in this study, it is considered with the ante mortem items.

high; one of 0.300 is moderate, and one which does not differ from zero by four times its probable error is not considered statistically significant. The relative significance of the coefficients in each of the lists can be evaluated on this basis. For convenience each list is divided into sections, consisting of coefficients below 0.300 and multiples of 0.100 above that level.

The coefficients of correlation obtained for heart weight in relation to other items are as follows:

	Heart-weight-coefficient
Range, over 0.500:	
Circumference of heart (over apex)	+0.538±0.031
Live weight	+.509±.031
Body-surface area	+.503±.031
Range, 0.400–0.499:	
Circumference of fore chest	+.494±.032
Circumference of rear chest	+.484±.032
Volume of barrel	+.471±.033
Weight of carcass	+.463±.033
Depth of rear chest	+.457±.033
Weight of hide	+.447±.034
Weight of liver	+.440±.035
Circumference of paunch	+.437±.034
Depth of fore chest	+.436±.034
Cross-section area of rear chest	+.431±.034
Depth (dorsoventral) of thoracic cavity	+.427±.036
Cross-section area of paunch	+.424±.034
Cross-section area of fore chest	+.422±.034
Circumference of heart at base	+.416±.036
Weight of spleen	+.415±.037
Length (anterior-posterior) of thoracic cavity	+.410±.037
Volume of thoracic cavity	+.400±.037
Range, 0.300–0.399:	
Weight of lungs	+.394±.038
Depth of paunch	+.392±.035
Height at withers	+.364±.036
Length of intestines	+.353±.037
Weight of blood	+.348±.037
Width of rear chest	+.348±.037
Length, withers to pinbones	+.341±.037
Width of paunch	+.324±.038
Width (lateral) of thoracic cavity	+.321±.040
Width of fore chest	+.315±.038
Range, 0.299 or less:	
Length, withers to hips	+.263±.039
Weight of pancreas	+.158±.043
Weight of empty stomach	+.140±.042
Weight of brain	+.124±.043
Thoracic index	−.113±.041
Wedge shape, depth ratio [1]	+.101±.041
Legginess	−.094±.042
Wedge shape, circumference ratio [1]	+.088±.042
Abdominal index	+.074±.042
Rear-chest index	−.058±.042
Wedge shape, width ratio [1]	+.022±.042

[1] For explanation of item, see p. 209.

Only three of the items in this list have coefficients above 0.500 and may be considered as highly correlated with weight of heart, but a number of items have coefficients closely approaching that level. Conspicuous among the highest coefficients are items that might be considered as very closely associated with the general bulk of the animal body, such as live weight, body-surface area, volume of barrel,

weight of carcass, and weight of hide. It is noteworthy that the three body circumferences and the three calculated cross-section areas—also somewhat closely associated with body bulk—all have coefficients above 0.400. Depths of chest and all measurements of the thoracic cavity except width have coefficients ranging between 0.400 and 0.499, showing a fairly significant correlation between the size of the heart and the cavity in which it is located.

The comparatively low coefficients ranging between 0.300 and 0.399 include all items of external and thoracic width; also height at withers and length from withers to pinbones, which are measurements of body scale. One other measurement of body length (withers to hips) has a coefficient below 0.300. The seven ratios and indexes that were calculated to show body proportion are all at the bottom of the list. Among the weights or measurements of the other internal organs in relation to the heart, weight of liver has the highest coefficient (0.440), and the rest have coefficients ranging down to that for weight of brain, which is only 0.124.

In general the weight of heart is rather highly correlated with live weight and the measurements associated with body bulk. It is moderately correlated with depth of chest and depth, length, and volume of the thoracic cavity, but only slightly correlated with body width and width of thoracic cavity, or with height and length of body which represent body scale. It is only moderately correlated with the weight or measurement of the other internal organs, except weight of liver, and for several of them (weights of pancreas, stomachs, and brain) the correlations are not significant. Moreover, it is not significantly correlated with any of the ratios or indexes calculated to represent body proportions.

The list of correlation coefficients obtained for weight of lungs in relation to other individual items is as follows:

	Lung-weight coefficient
Range, over 0.400:	
Volume of barrel	+0.444±0.035
Length, withers to hips	+.437± .036
Depth of fore chest	+.432± .036
Length, withers to pinbones	+.423± .036
Body-surface area	+.418± .036
Depth of rear chest	+.418± .036
Volume of thoracic cavity	+.404± .039
Length (anterior-posterior) of thoracic cavity	+.400± .039
Range, 0.300–0.399:	
Weight of heart	+.394± .038
Depth (dorsoventral) of thoracic cavity	+.383± .040
Depth of paunch	+.379± .038
Circumference of fore chest	+.372± .038
Live weight	+.343± .039
Weight of liver	+.340± .040
Weight of hide	+.340± .039
Circumference of rear chest	+.335± .039
Cross-section area of rear chest	+.333± .039
Cross-section area of fore chest	+.321± .040
Cross-section area of paunch	+.313± .040
Circumference of paunch	+.307± .040
Height at withers	+.303± .040
Width (lateral) of thoracic cavity	+.302± .042
Length of intestines	+.300± .040
Range, under 0.299:	
Weight of carcass	+.295± .040
Weight of spleen	+.295± .042
Weight of blood	+.264± .041

Range, under 0.299—Continued.	*Lung-weight coefficient*
Weight of empty stomach	+0.252±0.041
Width of paunch	+.214± .042
Width of rear chest	+.211± .042
Width of fore chest	+.200± .042
Circumference of heart over apex	+.188± .045
Circumference of heart at base	+.180± .045
Weight of brain	+.160± .044
Legginess	−.129± .043
Rear-chest index	+1.090± .044
Weight of pancreas	+.057± .045
Wedge shape, depth ratio [1]	+.043± .044
Wedge shape, circumference ratio [1]	+.031± .044
Abdominal index	+.027± .044
Thoracic index	+.011± .044
Wedge shape, width ratio [1]	+.002± .044

[1] For explanation of item, see p. 209.

In most cases the lung-weight coefficients are considerably lower than those for heart weight, the highest coefficient for lung weight being 0.444, as compared with a maximum of 0.538 for heart weight. The lung-weight coefficients, like those for heart weight, are comparatively high for volume of barrel and body-surface area but the other items discussed in connection with the heart-weight list as being associated with body bulk (live weight, weight of carcass, weight of hide, body circumferences, and cross-section areas) all have coefficients below 0.400, including many only slightly above 0.300 and one (for weight of carcass) only 0.295. The coefficients for volume and length of thoracic cavity are above 0.400, and that for depth of thoracic cavity is 0.383, showing a fairly significant correlation between size of lungs and size of the cavity in which they are located.

For some unexplained reason, the coefficients for body lengths in relation to lung weight are near the top of this list and are much greater in magnitude than those obtained in relation to heart weight (0.437 and 0.423 for lung weight as compared with 0.263 and 0.341 for heart weight). Despite this fact, height at withers—a measurement previously associated with body length as representing body scale—has a coefficient of only 0.303 for weight of lungs, which is even lower than that for weight of heart. Coefficients of correlation for body depths with lung weight, though slightly lower in magnitude than with heart weight, are somewhat higher in this list. Here, again, the coefficients for depth of body and for depth of thoracic cavity are definitely greater in magnitude than those for width of body. The seven ratios and indexes showing body proportions are all located in the eight lowest positions and none has a coefficient showing any significance.

Among the coefficients based on the lung weight in relation to other internal organs, that for weight of liver ranks higher than those for the weights or measurements of the internal organs listed except the heart, but neither the liver nor the heart has more than a moderately significant correlation with lung weight. Length of intestines (0.300) has the next highest coefficient of the internal-organ items, the rest having coefficients either of doubtful significance or of no significance.

In general, there is some indication of a moderate correlation between lung weight and certain measurements of body surface, body

volume, and measurements of the thoracic cavity. The reason for the lung weight having a much lower coefficient than the heart weight (0.343 and 0.509, respectively) when correlated with live weight is not easily explained, except on the basis that the heart might be expected to have a weight more nearly in proportion to the mass of the body because it is a muscular organ. Coefficients for depth—both external and internal—are definitely higher than the corresponding ones for width. Body proportions as shown by indexes and ratios have no relation to size of lungs. Among the internal organs only the weight of heart—its companion in the thoracic cavity—and the weight of liver and length of intestines are even moderately correlated with weight of lungs. Taken as a whole, the weight of lungs cannot be considered as highly correlated with any of the ante-mortem or post-mortem items studied though some moderately significant coefficients are shown.

The list of correlation coefficients obtained for length of intestines in relation to other items is as follows:

	Intestine-length coefficient
Range, 0.300 and over:	
Weight of empty intestines	+0.387±0.035
Width (lateral) of thoracic cavity	+.353± .037
Weight of heart	+.353± .037
Volume of thoracic cavity	+.337± .038
Length (anterior-posterior) of thoracic cavity	+.301± .038
Weight of lungs	+.300± .040
Range, under 0.300:	
Depth of rear chest	+.287± .036
Live weight	+.268± .036
Weight of liver	+.255± .041
Weight of spleen	+.255± .041
Cross section area of rear chest	+.254± .037
Depth of fore chest	+.252± .037
Body-surface area	+.252± .037
Circumference of fore chest	+.251± .037
Depth (dorsoventral) of thoracic cavity	+.250± .039
Circumference of rear chest	+.250± .037
Weight of carcass	+.244± .037
Weight of hide	+.243± .037
Width of loin	+.240± .037
Depth of paunch	+.236± .037
Circumference of paunch	+.231± .037
Cross-section area of paunch	+.228± .037
Width of hips	+.227± .037
Weight of pancreas	+.218± .040
Cross-section area of fore chest	+.213± .037
Volume of barrel	+.202± .038
Height at withers	+.200± .038
Length, withers to pinbones	+.199± .038
Length, withers to hips	+.192± .038
Width of rear chest	+.176± .038
Width of paunch	+.173± .038
Length of loin	+.168± .038
Weight of empty stomach	+.142± .039
Width of fore chest	+.122± .039
Weight of blood	+.113± .039
Legginess	−.071± .039
Wedge shape, width ratio [1]	+.068± .039
Weight of brain	+.061± .040
Wedge shape, depth ratio [1]	+.057± .039
Wedge shape, circumference ratio [1]	+.053± .039
Thoracic index	−.035± .039
Abdominal index	−.019± .039
Rear-chest index	+.004± .039

[1] For explanation of item, see p. 209.

A comparison of this list with the two former lists shows that the correlation coefficients for length of intestines are definitely lower than those for either heart weight or lung weight; in fact, only 6 of 43 items have coefficients above 0.300 and can be classed as being even moderately significant. Width of thoracic cavity is near the top of the list, which is surprising since body widths were not significant in the other studies. The fact that five of the six items having coefficients above 0.300 are weights of thoracic organs or measurements of the thoracic cavity, is not easily explained.

Length of intestines does not appear to be significantly correlated with body weight or with the measurements of body bulk—not even volume of barrel. Moreover, it does not seem to be correlated with the external measurements of the paunch to any greater extent than with those of the chest. In fact, there is some indication that the reverse is true, though the differences are too slight and the coefficients too low to have much significance. Length of intestines is in no sense correlated with body scale, as body height and body lengths all have coefficients falling between 0.200 and 0.192. In this list, as in that for lung weight, the seven ratios and indexes representing body proportions are all located in the eight lowest places in the list. Attention is called to the fact that the ratios showing degree of wedge shape, which is commonly thought of as being associated with feeding capacity, all have coefficients ranging from 0.068 to 0.053.

These results cast considerable doubt on the possibility of efficiently judging the feeding capacity of cows on the basis of their external body measurements, for two reasons. One is the low magnitude of all the coefficients obtained in relation to length of intestines, and the other is the fact that the measurements of body mass, surface area, barrel capacity, body scale, and wedge shape nearly all have coefficients lower than those for measurements of thoracic cavity and weight of thoracic organs.

The correlation coefficients obtained for live weight in relation to other items are as follows:

	Live-weight coefficient
Range, 0.900 and over:	
Body-surface area	+0.939±0.004
Range, 0.800–0.899:	
Volume of barrel	+.838± .011
Circumference of fore chest	+.821± .012
Range, 0.700–0.799:	
Circumference of rear chest	+.788± .014
Weight of carcass	+.771± .015
Cross-section area of paunch	+.762± .015
Circumference of paunch	+.750± .016
Cross-section area of rear chest	+.745± .016
Depth of fore chest	+.721± .017
Cross-section area of fore chest	+.719± .017
Depth of rear chest	+.711± .018
Range, 0.600–0.699:	
Depth of paunch	+.680± .019
Width of paunch	+.676± .019
Weight of hide	+.664± .020
Width of hips	+.651± .020
Width of rear chest	+.650± .021
Volume of thoracic cavity	+.601± .026

	Live-weight coefficient
Range, 0.500–0.599:	
Length, withers to pinbones	+0.586±0.023
Width of fore chest	+.586± .023
Height at withers	+.572± .024
Weight of heart	+.509± .031
Length, withers to hips	+.508± .026
Weight of liver	+.504± .032
Range, 0.400–0.499:	
Width of loin	+.491± .027
Length (anterior-posterior) of thoracic cavity	+.486± .031
Weight of blood	+.484± .027
Weight of empty stomach	+.412± .032
Range, 0.300–0.399:	
Weight of empty intestines	+.376± .035
Weight of lungs	+.343± .039
Depth (dorsoventral) of thoracic cavity	+.325± .036
Width (lateral) of thoracic cavity	+.310± .037
Width of head	+.301± .032
Range, under 0.299:	
Abdominal index	−.275± .033
Length of intestines	+.268± .036
Length of loin	+.262± .034
Legginess	−.257± .033
Weight of spleen	+.247± .041
Rear-chest index	−.236± .034
Wedge shape, depth ratio [1]	+.236± .034
Thoracic index	−.232± .034
Wedge shape, circumference ratio [1]	+.191± .034
Weight of pancreas	+.172± .040
Wedge shape, width ratio [1]	+.117± .035
Weight of brain	+.066± .038

[1] For explanation of item, see p. 209.

The coefficients obtained for many items when correlated with live weight are higher than anticipated. Among the 125 given in the three previous lists, for heart weight, lung weight, and length of intestines, only 3 were above 0.500 and could be considered as high. These three all pertain to heart weight. The maximum coefficient in the lung-weight list was 0.444 and that in the intestine-length list was only 0.387. Live weight was found to have correlation coefficients above 0.500 for 23 of the 44 items studied. Practically all of the 23 items, however, were items indicating body dimensions or body bulk. Only two were items showing size of internal organs—namely, the heart and the liver.

A large cow, particularly a bulky cow, is almost inevitably a heavy one. As a consequence, the measurements of mass or bulk would almost certainly have high coefficients of correlation with live weight, and it is not surprising to find body-surface area, volume of barrel, weight of carcass, and all the circumferences and cross-section areas of the fore chest, rear chest, and paunch in the highest sections of the list (all above 0.700). The location of body-surface area (0.939) at the top of the list is to be expected, since live weight is used as a factor in calculating body-surface area. The body depths all have coefficients of very high magnitude, ranging from 0.680 upwards, and the coefficients for body widths are much more significant in the live-weight correlations than in those for heart weight, lung weight, and intestine length.

The measurements of length and height, previously discussed as representing body scale, have rather highly significant coefficients (all

above 0.500), whereas in the previous considerations, they were definitely less significant. The coefficients for measurements of thoracic cavity, although moderately or definitely significant, are more remote from the top in the live-weight list than in the other lists. Coefficients for these measurements are even lower than those for volume of thoracic cavity, which has the high coefficient of 0.601. This may appear to be contradictory, but it probably means that the total volume of the thoracic cavity increases with greater body size without regard to any given dimension of the thoracic cavity, that is, of length, width, or depth. The increased volume may come from an increase in one of these dimensions in some cases, from an increase in another dimension in other cases, or from an increase in all these dimensions in still other cases. None of the ratios or indexes is significantly correlated with live weight. Width of head and length of loin are not very significantly correlated with increased live weight, and legginess has a very moderately significant negative correlation.

As stated, the only internal organs having coefficients above 0.500 are weight of heart and weight of liver. Weights of stomach, intestines, and lungs have moderately significant coefficients, but length of intestines and weights of spleen, pancreas, and brain appear to have little relation to weight of body. In fact the brain has the lowest coefficient (0.066) of the 44 items.

One particularly important point is the fact that the single body measurement having the highest correlation coefficient (0.821) is circumference of fore chest, a measurement employed in Denmark and used for generations in some sections of the United States as a basis for estimating live weight in cattle. A compilation of data by the Bureau of Dairy Industry has provided a table [9] from which the live weight of dairy cattle can be estimated from the heart girth with a very high degree of accuracy. From this table a tapeline has been prepared which permits a direct reading of the live weight when the tapeline is passed around the body in the region of the fore chest. Similar tables [10] have since been prepared by the Bureau of Animal Industry for estimating the live weight of beef and dual-purpose cattle on the same basis. Attention is also called to a table for estimating live weight from heart girth, prepared by Ragsdale and Brody (*13*) and to one prepared by Brody, Davis, and Ragsdale (*2*), which includes the data obtained at the Missouri and Nebraska agricultural experiment stations.

In general, the body measurements—particularly those that represent bulk, magnitude, and scale—are highly correlated with live weight. The only internal organs whose weights are highly correlated with live weight are liver and heart. There is no relation between weight of brain and live weight. The coefficients for legginess and for relation of depth to width of body are all negative but of low magnitude. They do, however, suggest a slight tendency for the short-legged broad animal to have greater body weight than one with long legs and a deep narrow body.

[9] KENDRICK, J. F., and PARKER, J. B. ESTIMATING THE WEIGHTS OF COWS FROM HEART-GIRTH MEASUREMENTS. U. S. Bur. Dairy Indus. BDIM-695, 2 pp. 1936. [Mimeographed.]

[10] KNAPP, BRADFORD, JR. A METHOD OF ESTIMATING THE WEIGHTS OF BEEF AND DUAL-PURPOSE CATTLE FROM HEART-GIRTH MEASUREMENTS. U. S. Bur. Anim. Indus. A. H. D. 24, 4 pp. 1937. [Mimeographed.]

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Comparison of Correlation Coefficients for Heart Weight, Lung Weight, Intestine Length, and Live Weight

In the discussions of the individual correlation coefficients presented in the foregoing lists, frequent reference has been made to the comparative magnitude and range of coefficients for different combinations of items. These combinations or groups differ according to the arrangement of the individual items in the list under consideration. A uniform method of grouping the different ante-mortem and post-mortem items was needed to permit a comparison of the extent to which the heart weight, lung weight, intestine length, and live weight of the cows studied was correlated with certain body dimensions and proportions (depths, widths, heights, etc.), and with the size of the organs and body parts that are associated with the various body functions. Accordingly, the ante-mortem items were divided into eight groups: (1) live weight, (2) body height, (3) body lengths, (4) body depths, (5) body widths, (6) body circumferences, (7) the calculated body-bulk values (body-surface area, volume of barrel, and the cross-section areas of fore chest, rear chest, and paunch), and (8) the calculated body-proportion values. The latter include legginess, thoracic, rear-chest, and abdominal indexes, and the ratios representing wedge shape as determined on the basis of depth, width, and circumference of body. The post-mortem items were classified in five groups: (1) body size, (2) nervous system, (3) circulation and respiration, (4) digestion, and (5) endocrine glands, as described in connection with the discussion of variation.

The mean or composite correlation coefficient for each group was determined as follows: The sum of the products obtained by multiplying each coefficient by the square root of its population was divided by the sum of the square roots of the populations, for all of the items included in the group. This is an empirical method of arriving at composite correlation coefficients for the respective groups, on a basis whereby each of the individual coefficients is weighted as to population. Although each of two of the ante-mortem classifications (live weight and body height) and one of the post-mortem groups (nervous system) contain only a single item, they are referred to as groups and the individual coefficient is referred to as a composite. A summary of the composite correlation coefficients for each ante-mortem and post-mortem group is given in table 3.

As an illustration of the data presented in table 3, it will be noted in the column under heart weight that live weight, which represents only one item, has a coefficient of correlation of 0.509. Since this is the highest coefficient under heart weight it is given a rank of 1. The second highest coefficient, 0.472, is that of body circumferences. This coefficient represents a composite of those for three items in the list of heart-weight correlations on page 215, as follows: Circumference of fore chest, coefficient 0.494; circumference of rear chest, coefficient 0.484; and circumference of paunch, coefficient 0.437. The third highest in rank of the composite coefficients for heart weight is that for the calculated body-bulk values, and the fourth highest is that for body depths. At the other extreme, the thirteenth or lowest composite coefficient of correlation with heart weight is that for the calculated body-proportion values; the twelfth is that for the nervous-system group, represented by the brain; the eleventh is that for the

digestion group; and the tenth is that for body lengths. It appears, therefore, that the items most likely to be associated with hearts of greater weight in cows are those which indicate body magnitude such as live weight, calculated body-bulk values, body circumferences, and body depths.

TABLE 3.—*Summary of the composite correlation coefficients for each functional group in each list and for all lists combined*

Group	Items in group	Heart weight		Lung weight		Intestine length		Live weight	
		Rank	Coefficient	Rank	Coefficient	Rank	Coefficient	Rank	Coefficient
	Number								
Ante-mortem:									
Live weight	1	1	0.509	5	0.343	2	0.268		
Body height	1	8	.364	7	.303	9	.200	4	0.572
Body lengths	[1] 2	10	.302	1	.430	11	.186	7	.454
Body depths	3	4	.429	2	.410	3	.258	3	.704
Body widths	[2] 3	9	.329	11	.208	10	.188	5	.559
Body circumferences	3	2	.472	6	.338	7	.244	2	.786
Calculated body-bulk values	5	3	.450	3	.366	8	.230	1	.801
Calculated body-proportion values	7	13	.079	13	.048	13	.044	11	.220
Post-mortem:									
Body-size group	6	7	.412	4	.353	1	.287	6	.535
Nervous-system group	1	12	.124	12	.160	12	.061	12	.066
Circulation-and-respiration group	[3] 4	5	.423	9	.259	5	.251	8	.449
Digestion group	[4] 4	11	.272	10	.238	6	.250	9	.345
Endocrine-gland group	1	6	.415	8	.295	4	.255	10	.247
Composite for all items represented [5]			.335		.270		.202		.502

[1] Length of loin added under intestine length and live weight.
[2] Widths of loin and hips added under intestine length; widths of loin, hips, and head added under live weight.
[3] Heart weight omitted under heart weight; lung weight omitted under lung weight; heart circumferences omitted under intestine length and live weight.
[4] Intestine length omitted and intestine weight added under intestine length; intestine weight added under live weight.
[5] Total number of items represented under heart weight, 41; under lung weight, 41; under intestine length, 43; and under live weight, 44.

Since heart weight has a high correlation with live weight, it is to be expected that the items which are indicative of heart weight would also be indicative of live weight, and for the most part this is true. There is considerable similarity in the ranking of the coefficients under the two headings, although a number of exceptions exist. The endocrine-gland group, which is represented by only one organ, the spleen, is more closely associated with heart weight than with live weight. The circulation-and-respiration group also has a higher rank in the heart-weight than in the live-weight column, probably because the heart circumferences are included in the former but not in the latter. On the other hand, the measurements of body height, body widths, and body lengths have higher ranks and are more indicative of live weight than they are of heart weight. The items in the digestion, nervous-system, and body-proportion groups seem to be a little more closely related to live weight than to heart weight. This is also true of their relationship to lung weight and to intestine length, except that the rank of the digestion group is highest in the intestine-length column. This is no doubt due in part to the fact that the intestine-length and intestine-weight coefficients are included in the group.

Many of the composite coefficients in the lung-weight column and most of the coefficients in the intestine-length column are too small to be significant. The great difference in relative ranking of some of the coefficients in these two columns from those in the heart-weight and live-weight columns, is interesting and perhaps significant. For instance, the composite coefficients for body lengths rank first under lung weight but have a low or medium rank under the other three heads. The composite coefficients for the body-depth measurements rank second for lung weight and third for intestine length. These ranks are equal to or higher than those for heart weight and live weight. Body circumferences, however, which ranked second with heart weight and with live weight, dropped to sixth and seventh with lung weight and intestine length. Body widths appear to be of comparatively little importance in relation to heart weight, lung weight, or intestine length but have a definitely higher correlation for live weight. Live weight and the post-mortem body-size group rank high among the coefficients for intestine length. Too much significance should not be placed on the relative ranking of these composite coefficients under intestine length, however, because of their low values. For instance, the highest ranking coefficient under intestine length is that for the body-size group, with a value of 0.287. The coefficient for this same group ranks seventh for heart weight and sixth for live weight, but has values of 0.412 and 0.535, respectively. Therefore, these sixth or seventh place coefficients of this group must be more indicative of heart weight and live weight than is the coefficient having first rank for this same group under intestine length.

It is difficult to explain why the live-weight group has a rank of 5 for lung weight, whereas for heart weight and intestine length its rank is 1 and 2, respectively. Lung weight appears to be more closely correlated with several of the skeletal measurements than with body weight.

The calculated body-bulk values (ante-mortem) are, as might be expected, closely correlated with live weight (rank 1). Both heart weight and lung weight also rank high for this group but length of intestines has a rank of 8, with the very low composite of 0.230.

The comparatively high ranks (2 to 4) for body depths in all cases may point to a comparatively high significance of body-depth measurements and their importance as a measure of body size and as an indicator of size of internal organs.

Body height—one of the measurements of scale—is fairly closely correlated with live weight, but has not only low coefficients but lower ranks (8, 7, and 9, respectively) for heart weight, lung weight, and intestine length.

The body widths are definitely less closely correlated with live weight than are body depths. The difference between the ranks for depth and for width is less for live weight than for heart weight, lung weight, or intestine length. Probably this is because an increase in width of body has a tendency to contribute directly to live weight through fat deposition, whereas depth of body is more nearly a measurement of skeletal size.

Some of the facts concerning the post-mortem, body-size group are not readily explainable. This group includes the weight of hide, weight of carcass, and the three dimensions and the calculated volume

of the thoracic cavity. Four of the six items are measurements of the thoracic cavity. Although the composite coefficient for this group listed under live weight, table 3, is of fairly high magnitude, it ranks sixth in the live-weight list despite the tendency for weight of carcass to raise the composite value. Apparently the size of chest cavity is not so closely correlated with live weight as are a number of the external body measurements that are more representative of body mass than of skeletal size. This is more specifically shown by the fact that the composite coefficient for the four thoracic measurement items alone in relation to live weight is 0.430. This value is lower than any of those listed in table 3 for the ante-mortem groups in the live-weight list, except the one for calculated body-proportion values. Moreover, composite coefficients based on the four thoracic measurement items considered as a group in relation to heart weight, lung weight, and length of intestines, are 0.390, 0.372, and 0.310, respectively. This fact indicates that the size of the heart is not as closely correlated with the size of the cavity in which it is located as with the ante-mortem groups for live weight, body depths, body circumferences, and calculated body-bulk values, or with three of the post-mortem groups, whereas the weight of the lungs, which also are housed in the thoracic cavity, is more highly correlated with the size of that cavity than with any other groups of external body or internal anatomical measurements except lengths and depths of body. Strangely, the composite coefficient of 0.310 for the four thoracic measurements in relation to length of intestines, which are located in the abdominal cavity, is higher than the composite for any of the ante-mortem or post-mortem groups listed in table 3 under intestine length.

Composite coefficients listed under live weight in table 3 are much higher for the ante-mortem than for the post-mortem groups, indicating that the external body measurements are more closely correlated with live weight than are the size of the internal organs or the measurements of the thoracic cavity. There is a tendency also for the composites listed under heart weight and lung weight to be of comparatively higher magnitude in the case of the ante-mortem groups, but the difference is negligible for length of intestines.

Composite coefficients shown in table 3 for the 41 heart-weight items, the 41 lung-weight items, the 43 intestine-length items, and the 44 live-weight items, indicate that on an average live weight is rather highly correlated, heart weight is moderately correlated, and lung weight and intestine length have little relation to body dimensions and anatomy in cows.

Comparison of Correlation Coefficients for Depths and for Widths of Fore Chest, Rear Chest, Paunch, and Thoracic Cavity

Although by no means the only result brought out or necessarily the one having the greatest importance from a practical viewpoint, one of the most consistent results shown is the comparatively high magnitude of correlation coefficients obtained for depths as compared with widths of body in relation to heart weight, lung weight, intestine length, and live weight. Because of the consistency of this result and the fact that similar results were reported by Becker (*1*), Seeberger (*15*), and Mierswa (*8*), it seemed desirable to determine additional

correlations and to test the consistency of the data when different combinations and greater numbers of items of external body dimensions and internal anatomy were included.

In the case of the fore-chest measurements, comparable coefficients have been determined for 11 items, and coefficients not strictly comparable are given for 2 other items. Composite coefficients also have been calculated for all groups which contain more than one item having comparable coefficients for depth and width. Since two comparable coefficients of correlation are given for each of the selected items, it was not possible in both cases to arrange the items in the order of descending magnitude of the coefficients. The items were grouped for convenience of comparison according to the previously used classification (pp. 222–223) based on the dimensions of the body and the functions of its parts. The results are shown in table 4.

TABLE 4.—*Comparison of the individual and group composite correlation coefficients for depth and for width of fore chest*

Item	Coefficient of correlation and probable error, for—		Extent to which coefficient for depth exceeds that for width
	Depth of fore chest	Width of fore chest	
Ante-mortem:			*Percent*
Live weight	0.721±0.017	0.586±0.023	23
Body height (at withers only)	.658± .020	.251± .033	162
Body-length group:			
Withers to hips	.722± .017	.106± .035	581
Withers to pinbones	.645± .021	.201± .034	221
Composite for group	.684	.153	347
Body circumference (at fore chest only)	.796± .013	.659± .020	21
Post-mortem:			
Body-size group:			
Weight of hide	.627± .022	.463± .028	35
Depth (dorsoventral) of thoracic cavity	[1] .611± .026		
Width (lateral) of thoracic cavity		[1] .423± .034	
Volume of thoracic cavity	.644± .024	.295± .038	118
Composite for group	.635	.385	65
Circulation and respiration group:			
Weight of blood	.446± .028	.296± .032	51
Weight of lungs	.432± .036	.200± .042	116
Weight of heart	.436± .034	.315± .038	38
Composite for group	.439	.273	61
Digestion group:			
Length of intestines	.252± .037	.122± .039	107
Composite for all comparable items	.587	.322	82

[1] Not comparable.

The coefficient for depth of fore chest exceeds the corresponding one for width of fore chest in the case of every one of the 11 individual items and of each group compared, confirming observations previously made. This excess is expressed as a percentage of the value of the width coefficient, in the last column of table 4. The relative degree of excess varies from 581 percent for length from withers to hips, to 21 percent for circumference of fore chest. It is noteworthy that the three highest individual differences all appear in connection with the items representing body height and length. Since these differences are the result of high coefficients for depth and low coefficients for width, they indicate that depth of body (fore chest) increases along

with increases in height and length of body, whereas width of body does not follow so closely. The two lowest differences are found in connection with live weight and circumference of fore chest—both measurements of body mass. All of the coefficients involved in this case are of high magnitude. Width of chest follows increases in live weight and chest circumference more closely than it follows increases in skeletal measurements. It appears that fleshing affects width of chest to a greater extent than it affects depth of chest. Length of intestines is not significantly correlated with either depth or width of fore chest. It appears that the measurements of body scale are highly correlated with those of depth but not with those of width of fore chest, that the size of the internal organs is not highly correlated with either depth or width of fore chest, and that the measurements of body mass (circumference of fore chest and live weight) are highly associated with both depth and width of fore chest.

In view of the high correlation between body (fore chest) width and circumference, the much lower coefficient for body width with weight of hide, which probably follows body surface quite closely, is rather surprising. This might be explained by the low correlation of body width with body length, which would be the other dimension in determining body surface, or by variations in the thickness of hide. The correlation between the exterior depth of chest and depth of thoracic cavity is high. The correlation between the exterior width of chest and width of thoracic cavity is significant, but is much lower than that for the corresponding depths. The correlation between exterior depth of chest and volume of thoracic cavity is even higher than that between the exterior depth and the depth of the cavity, but the correlation between exterior width and volume of the thoracic cavity is much lower than that between exterior width and interior width. This probably indicates that for thoracic cavity, length is a more important factor than width in determining its volume. It may also explain the relatively high correlation shown between body lengths and lung weight (table 3) and between length of thoracic cavity and lung weight (p. 216).

In order to determine whether the same ratio or percentage of difference between coefficients for depth and width that was shown for measurements of the fore chest would prevail in connection with measurements of the rear chest, paunch, and thoracic cavity, coefficients of correlation for these additional measurements with heart weight, lung weight, intestine length, and live weight were calculated. The results are given in table 5.

The depth (dorsoventral) and width (lateral) measurements of the thoracic cavity were taken at a plane intermediate between the planes used in obtaining external measurements of depth and width of fore chest and rear chest. Depth and width of thoracic cavity are particularly suitable for study because their accuracy was not, like some ante-mortem measurements, dependent on the standing position of the animal. The carcasses were always in the same position (suspended) when the measurements were obtained. Moreover, the thoracic measurements are virtually skeletal dimensions of the chest cavity and are not greatly affected by fleshing or fat deposition.

TABLE 5.—*Comparison of the correlation coefficients for depth and for width of fore chest, rear chest, paunch, and thoracic cavity*

Item	Fore chest			Rear chest		
	Depth coefficient	Width coefficient	Relation of width to depth coefficient	Depth coefficient	Width coefficient	Relation of width to depth coefficient
			Percent			*Percent*
Live weight	0.721	0.586	81.3	0.711	0.650	91.4
Heart weight	.436	.315	72.2	.457	.348	76.1
Lung weight	.432	.200	46.3	.418	.211	50.5
Intestine length	.252	.122	48.4	.287	.176	61.3
Composite coefficient	.469	.316	67.4	.476	.358	75.2

Item	Paunch			Thoracic cavity		
	Depth coefficient	Width coefficient	Relation of width to depth coefficient	Depth coefficient	Width coefficient	Relation of width to depth coefficient
			Percent			*Percent*
Live weight	0.680	0.676	99.4	0.325	0.310	95.4
Heart weight	.392	.324	82.7	.427	.321	75.2
Lung weight	.379	.214	56.5	.383	.302	78.9
Intestine length	.236	.173	73.3	.250	.353	141.2
Composite coefficient	.431	.360	83.5	.344	.322	93.6

All four individual items have coefficients which are greater for depth than for width of fore chest, of rear chest, and of paunch, although the difference for live weight in the latter comparison is too small to be of any significance. Correlation coefficients are greater for depth than for width of thoracic cavity for three of the four items compared, the only exception occurring in connection with length of intestines. A study of the weight of heart and weight of lungs (both thoracic organs) in relation to the depth and width of fore chest, rear chest, and thoracic cavity (all thoracic measurements) shows composite coefficients of 0.434 and 0.259, respectively, for depth and width of fore chest; 0.438 and 0.281 for depth and width of rear chest; and 0.405 and 0.312 for depth and width of thoracic cavity. Although the difference is not as great for the thoracic cavity as for the external chest measurements, it is sufficiently great to indicate a definite tendency for the thoracic organs to be more highly correlated with depth than with width of chest.

There is a definite tendency for the difference in the magnitude between the correlation coefficients for depth and for width to be less for measurements at the rear chest than at the fore chest and still less for those at the paunch, when these measurements are studied in relation to live weight, heart weight, lung weight, and intestine length.

With respect to body measurements, in the case of live weight the correlation coefficient for width of body is 81.3 percent as great as that for depth measured at the fore chest, 91.4 percent at the rear chest, and 99.4 percent at the paunch. In the case of heart weight, the coefficient for width is 72.2 percent as great as that for depth when

measured at the fore chest, 76.1 percent as great when measured at the rear chest, and 82.7 percent as great at the paunch. The corresponding percentages are 46.3, 50.5, and 56.5, respectively, for lung weight, and 48.4, 61.3, and 73.3, respectively, for intestine length. These differences appear to be too great to be accounted for by changes that might be brought about by the position in which the animal was standing when measured, or by differences in deposition of fat.

The coefficients of correlation for live weight with thoracic-cavity depth and width in table 5 are less than half as great as those for the exterior body measurements, and the coefficient for width is almost as great as that for depth. In respect to heart weight, the coefficients of correlation with measurements of the thoracic cavity do not differ greatly in magnitude from those obtained for the exterior measurements, and the relationship of the coefficient for width of thoracic cavity to that for depth is about the same as those for the fore-chest and rear-chest measurements. In the correlation of lung weight with thoracic cavity, the coefficient for width is greater than the width coefficient for any of the three exterior measurements, and the percentage relationship of the coefficient for width of thoracic cavity to that for depth of the cavity is much greater than that for any of the exterior depths and widths. In the correlations with intestine length, the coefficient for depth of thoracic cavity is not greatly different from those for depth of fore chest, rear chest, and paunch, but the coefficient for width of thoracic cavity is much greater than that for any other depth or width in the series for intestine length, and is the only one that is large enough to be significant. The coefficient for width of thoracic cavity is 141.2 percent as great as that for depth of thoracic cavity.

RESULTS FOR THE DIFFERENT BREEDS

Up to this point the study has dealt with the combined data for all breeds. Analysis of the data in this way, although not ideal, seemed warranted, inasmuch as many of the cows could not be definitely identified with any specific breed and to divide those remaining into groups would too greatly limit the number of data for some of the breeds. However, to determine whether the results obtained by studying the combined data were representative for the different breeds, the data were divided into breed groups. Only those cows that showed definite characteristics of some breed were used in this phase of the study. On account of the small number of animals considered to be Jerseys, Guernseys, and Ayrshires, and their similarity in size, the cows of these three breeds were combined in one breed group to be compared with the Holsteins and the Shorthorns, which appeared in fairly large numbers. Correlation coefficients for depth of fore chest and for width of fore chest with heart weight and lung weight are presented in table 6.

There appear to be some significant differences in the breeds as grouped in table 6. In the coefficients of correlation for heart weight with depth and width of fore chest, the coefficient for width of fore chest is 92.7 percent as great as that for depth of fore chest in the Holsteins, but it is only 55.2 percent as great in the Shorthorns, and 64.5 percent as great in the Ayrshire-Guernsey-Jersey group. The correlation between heart weight and depth of fore chest is highest for the

Ayrshire-Guernsey-Jersey group and lowest for the Holsteins. The correlation between heart weight and width of fore chest is greatest for the Holsteins and least for the Shorthorns.

TABLE 6.—*Correlation coefficients for depth and width of fore chest, studied in relation to heart and lung weight, and divided by breeds*

Item	Holstein				Shorthorn				Ayrshire-Guernsey-Jersey			
	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient
	Number			*Percent*	*Number*			*Percent*	*Number*			*Percent*
Weight of heart	114	0.450	0.417	92.7	54	0.460	0.254	55.2	45	0.519	0.335	64.5
Weight of lungs	97	.362	.217	59.9	47	.364	.324	89.0	44	.489	.229	46.8

In the correlations between lung weight and depth and width of fore chest, the coefficient for width is 59.9 percent as great as that for depth in the Holsteins, 89.0 percent as great in the Shorthorns, and only 46.8 percent as great in the Ayrshire-Guernsey-Jersey group. The correlation between lung weight and depth of fore chest is greatest for the Ayrshire-Guernsey-Jersey group; coefficients for the Shorthorns and the Holsteins are much lower and on very nearly the same level. The correlation with width of fore chest is greatest for the Shorthorns; coefficients for the Ayrshire-Guernsey-Jersey and Holstein groups are considerably lower and on approximately the same level. No explanation is offered for these apparent differences in the breed groups. It seems possible that they would be modified to some extent if larger numbers were available for the correlations.

Since the breed study was undertaken primarily to determine whether or not the breed data followed the pattern of the combined data, reference is made to the results of that comparison. Although a number of breed differences are apparent, the coefficient for depth is greater than the corresponding one for width of fore chest in each of the six comparisons shown in table 6. Moreover, the heart-weight coefficient exceeds the corresponding lung-weight coefficient in five of the six comparisons. Composite coefficients for heart and for lungs are 0.409 and 0.325 respectively. Comparative composites for heart weight and for lung weight based on the combined data were 0.376 and 0.316 when only the measurements of fore chest were included, and 0.335 and 0.270, respectively, when the comparison was based on all of the 40 items studied in relation to weight of heart and lungs. Despite the breed differences noted, therefore, the results obtained were generally similar whether the study was based on data for separate breed groups or on the combined data from all of the animals.

SUMMARY

The results here reported are confined to studies of the relationship between external form and internal anatomy since information regard-

ing the producing capacity of the cows included in this study was not available.

In addition to live weight and thickness of hide, the ante-mortem items studied include 17 measurements of skeletal size, 5 calculated values representing body bulk, and 10 calculated values showing body proportions. The post-mortem items include 19 weights or measurements showing size of organ or body part, 2 ratios showing the relation of heart and lung weight to live weight, and 1 value calculated to give an approximate measure of the volume of the thoracic cavity.

In both the ante-mortem and post-mortem data the arithmetic average and the median are almost equal for each individual item, indicating a normal distribution of data centering close to the average. The cattle selected for study apparently constitute a representative sampling.

The coefficients of variation for the calculated values representing body bulk are on an average greater than for any group of actual measurements of skeletal dimensions. The calculated body-proportion values show an even greater average variation; of these, the coefficients for wedge shape based on differences in measurement of fore chest and paunch have the highest average. The greatest variation in the group representing skeletal size is for length of loin, followed by the five measurements for width of body. On an average, variations for the ante-mortem measurements of skeletal size are much greater for width than for circumferences, depths, lengths, or height of body. Aside from the great variation in thickness of hide and in length of loin, the greatest variations are associated with measurements most likely to be affected by condition of fleshiness and fill, and the least with measurements representing skeletal size or body scale. The highest average variations for the post-mortem items occur in the group of visceral fats, the second highest in the group of digestive organs, and the lowest in the nervous-system group, represented by the brain, and the body-size group, which includes weight of hide, weight of dressed carcass, and carcass measurements. The pancreas has the highest individual variation and the three measurements of the thoracic cavity the three lowest. The coefficients for the heart and lungs are much larger than those for the individual dimensions of the thoracic cavity. Variations both for the individual items and for functional groups are much greater for the post-mortem than for the ante-mortem items. It appears that even with animals of the same body size and shape there may be marked differences in internal anatomy.

A total of 169 individual correlation coefficients have been determined to show the relation of heart weight, lung weight, intestine length, and live weight to other ante-mortem and post-mortem items and to each other. Additional determinations show the relation between depth and width of fore chest, rear chest, paunch, and thoracic cavity, and certain individual ante-mortem body dimensions and post-mortem weights and measurements. These studies are based on the combined data from all the animals included in the study. A separate study, in which the data are separated into breed groups, has been made to determine to what extent some of the results obtained from analyzing the combined data are supported by the data for the different breeds.

Heart weight is rather highly correlated with live weight and with the measurements associated with body bulk, such as body-surface

area, volume of barrel, body circumferences, weight of carcass, and weight of hide. Heart weight is moderately correlated with depth of chest, and with depth, length, and volume of the thoracic cavity in which it is located, but it appears to be only slightly correlated with width of body, width of thoracic cavity, or with height and length of body, which represent body scale. Only moderate correlations exist between heart weight and the weight or measurement of other internal organs except weight of liver, and for several of them (weights of pancreas, stomach, and brain) the correlations are of no significance. Heart weight is not significantly correlated with any of the ratios or indexes calculated to represent body proportions.

In general the individual correlation coefficients for lung weight are definitely lower than those for heart weight. The lung-weight correlations, like the heart-weight correlations, are comparatively high for volume of barrel and body-surface area, but correlations for the other body-bulk items are at best only moderate. A fairly significant correlation appears to exist between weight of lungs and the depth, length, and volume of the thoracic cavity in which they are located. Body lengths not only appear near the top of the list of lung-weight correlations, but their coefficients are actually greater in magnitude than those for heart weight. Despite this fact, body height—another measurement of body scale—has an even lower coefficient for lung weight than for heart weight. The lung weight has a much lower coefficient than the heart weight (0.343 and 0.509 respectively) when correlated with live weight. Lung weight is definitely more highly correlated with depth than with width of body or of the thoracic cavity. There is no significant relation between lung weight and the ratios and indexes that indicate body proportions. Aside from moderate coefficients for heart and liver weights, there is no significant correlation between weight of lungs and the size of other internal organs. Lung weight cannot be considered as highly correlated with either the ante-mortem or the post-mortem items studied, though some moderately significant coefficients are shown.

The correlation coefficients are definitely lower for length of intestines than for either heart weight or lung weight, only 6 of the 43 items studied being above 0.300. Length of intestines does not appear to be significantly correlated with body weight or the measurements of body bulk—not even volume of barrel. There is no significant correlation for the items representing body scale or body proportions, although the latter include wedge shape which is commonly thought of as being associated with capacity for feed consumption. Length of intestines appears to be more highly correlated with external measurements of the chest than with those of the paunch, although the differences are too small to have much significance. Most surprising of all findings is the fact that intestine length is more closely correlated with the weights of the thoracic organs and the width, volume, and length of the thoracic cavity, than with live weight, body bulk, body scale, or with abdominal or any other body measurements. This fact, together with the low magnitude of all of the intestine-length coefficients, casts considerable doubt on the possibility of efficiently judging the feeding capacity of cows on the basis of their external conformation.

Comparatively high correlations were anticipated for live weight, but the magnitude of the coefficients exceeded expectations. Among the 125 coefficients for heart weight, lung weight, and intestine length

only 3 were above 0.500, yet 23 of the 44 live-weight coefficients were above that level. Practically all of these, however, were for items indicating body dimensions or body bulk, only 2 of the 23 items being for internal organs. The highest coefficients are found in connection with body-surface area and volume of barrel, and nearly all the measurements of body bulk have coefficients above 0.700. The single body measurement having the highest coefficient (0.821) is for circumference of fore chest or "heart girth," a measurement employed for generations in Denmark and in some sections of the United States as a basis for estimating live weight in cattle. Body widths have lower coefficients than body depths though both are high. Measurements of body scale also are rather highly significant. Volume of thoracic cavity is highly correlated and length of thoracic cavity is more definitely correlated with live weight than either depth or width, though all the dimensions of the cavity are less significantly correlated than is volume. The only internal organs whose weights are highly correlated with live weight are heart and liver. There is no relation between weight of brain and live weight. Degree of wedge shape does not appear to be associated with live weight. The live-weight coefficients indicate that a heavy animal is almost certain to have, as is to be expected, a large surface area, a large volume of barrel, and large circumferences. Such an animal may be expected to have a correspondingly large heart and liver, and will also probably have a large quantity of blood, large stomachs, large lungs, and heavy intestines. There is little likelihood that the intestines or the loin will increase in length proportionately, and very little probability that any marked relationship will exist between live weight and weights of spleen, pancreas, or brain.

When the individual ante-mortem and post-mortem items were uniformly grouped on the basis of body dimensions or function of internal organs and body parts, the composite correlation coefficients for the various groups showed a wide range of variation in magnitude. The ranking of the groups on the basis of the magnitude of their composite coefficients is in some respects similar for heart weight, lung weight, and intestine length to that for live weight. One of the most noteworthy differences is the very high rank of body lengths for lung weight. In fact lung weight appears to be correlated more closely with a number of the skeletal measurements than with live weight. Other striking differences are the low rank of the body-bulk values, and the very high rank of the body-size group consisting largely of thoracic measurements, for intestine length. Body circumferences have high ranks for heart weight and live weight but lower values for lung weight and intestine length. The endocrine-gland group (spleen) has a very low rank for live weight though the coefficient is only slightly lower than for intestine length, where it ranks fourth. Ranks for body-proportion values and for brain weight are consistently low. Those for body widths are low except for live weight. Body depths have consistently high ranks—a fact which may point to the significance of depth of body as one of the most reliable indications of live weight and of size of internal organs.

Size of thoracic cavity does not appear to be as closely correlated with live weight as are a number of the external body measurements that are more representative of body mass than of skeletal size. The weight of heart is not as closely correlated with the size of the thoracic

cavity as live weight and many of the external body measurements. The weight of lungs, however, is more highly correlated with size of thoracic cavity than with any other group except lengths and depths of body. Length of intestines is more highly correlated with measurements of the thoracic cavity than with any of the ante mortem or post mortem groups.

It appears that, in general, live weight is rather highly correlated, that heart weight is moderately correlated, and that lung weight and intestine length have comparatively little relation to body dimensions and anatomy in cows.

The measurements of body scale (height and length of body) appear to be highly correlated with those for depth, but not with those for width of fore chest; the size of the internal organs is not highly correlated with either depth or width of fore chest, and the measurements of body mass (circumference of fore chest and live weight) are highly correlated with both depth and width of fore chest.

The correlation for exterior depth of chest and volume of thoracic cavity is high, even higher than that between the exterior depth of chest and depth of the cavity, but the correlation between exterior width and volume of the thoracic cavity is much lower than that for the exterior width and the interior width, probably indicating that length of the thoracic cavity is a more important factor in the volume of the thoracic cavity than is width.

In correlations of depth and width of fore chest, rear chest, and paunch with live weight, heart weight, lung weight, and intestine length, the magnitude of the correlations for width, as compared with those for depth, increases as the measurements progress from fore chest to paunch.

The data were divided into three breed groups: (1) Holsteins, (2) Shorthorns, and (3) Jerseys, Guernseys, and Ayrshires to determine whether or not certain results obtained with the combined data would hold for breeds differing widely in size and form. Because of the consistently marked difference between correlations for depth and width of chest these correlations were used as a basis for testing the data. While the results of the breed study substantiate those obtained from analysis of the combined data in showing a greater magnitude of correlation coefficients for depth than for width of fore chest, and higher coefficients for heart than for lungs, the study appears to indicate that there may be some significant breed differences. These suggested differences will be further explored when the more numerous data for the different breeds now being obtained for the purpose of determining the relationship of external form and size of internal organs to producing ability, are analyzed.

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